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# CLINICAL AND EXPERIMENTAL

## STUDIES IN TOXICOLOGIC CHEMISTRY*

### I. THE DETECTION OF THE OPIUM ALKALOIDS BY SELENIOUS-SULPHURIC ACID: THE SPECIFICITY OF THIS REAGENT FOR THE PHENOLIC GROUP

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WHEN one runs through the pages of a text devoted to toxicology, he is at once struck by the very great number of tests that have been devised for the toxic compounds usually met with in cadaveric material. These tests have been at times offered as legal evidence. Yet, in spite of their importance in forensic medicine, many of these have been inadequately investigated and have not been closely scrutinized as to their limitations and as to their specificity. To cite an example: A Portuguese physician, Urbino de Freitas, was accused of having poisoned three of his wife's nephews and of having brought about the death of one of them by poison given in enemata. The case was tried in 1893. The symptoms were those caused by opium in part only.

The experts for the prosecution reported the presence of morphine, narcein and delphinin in the tissues. They relied for their evidence as to the presence of these alkaloids upon a positive test with iodic acid, with Froehde's reagent, and with Lafon's reagent consisting of a mixture of selenious and sulphuric acids. It is now well known that neither the reduction of iodic acid nor the reduction of the molybdenum compound in Froehde's reagent is specific for the alkaloids mentioned, since hosts of organic compounds of biologic and non-biologic occurrence also have the power of reducing. The third reagent is highly recommended by Mecke, Autenrieth and others, and is considered specific for the alkaloids of the morphine group. In the course of this research we shall prove that the selenious-sulphuric reaction, while being very sensitive for these

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alkaloids, is also given by phenols. The opium alkaloids, therefore, react by reason of the phenolic group or groups present in the molecule. In the light of forensic medicine, it is of great importance to eliminate the presence in the tissue of such phenols, as phenol, cresol, tyrosin, hydroquinone, pyrocatechin, adrenalin and others, before ascribing a positive reaction to the presence of the opium alkaloids.

The first one to report the use of selenium as an alkaloidal reagent was Brandt. In 1875 he described a number of reactions obtained by a reagent containing concentrated sulphuric acid to which had been added a small quantity of selenic acid. Morphine and narcotine yielded characteristic color reactions. Morphine gave at first a bluish green, then a grass green, which quickly changed into reddish yellow, red and brown. Narcotine gave a blue color, which finally became green.

In 1885 Lafon reported a color reaction for morphine and codeine involving the use of a salt of selenious acid. Upon adding these alkaloids to ammonium selenite dissolved in sulphuric acid he obtained a magnificent green color. Sodium selenate he found also effective, although the color did not appear as quickly, nor was it as intense as that obtained with the selenite.

In 1891 Ferreira da Silva examined a number of alkaloids with the Lafon reagent and found that it permitted the characterization of berberin, eserin, narcein, narcotine, papaverine and solanin. Mecke, in 1899, reported experiments with an alkaloidal reagent consisting of 0.5 gram selenious acid dissolved in 100 grams of pure concentrated sulphuric acid. He recommended its use in the detection of the alkaloids found in opium. His results with these and with other compounds are given in tabular form.

TABLE I

| ALKALOID | REACTION IN THE COLD | REACTION ON HEATING |
|---|---|---|
| Acontin | colorless | dark brown violet |
| Apomorphine | dark blue violet | gradually turns dark brown |
| Atropine | colorless | almost colorless |
| Brucin | yellowish red | citron green |
| Caffeine | colorless | colorless |
| Cocaine | colorless | rose yellow |
| Codeine | blue, quickly changing to emerald green, and later to persistent olive | steel blue, then brown |
| Colchicin | intense citron yellow | yellow-brown |
| Conium | colorless | colorless |
| Delphinin | deep brown | brown |
| Digitalin | yellow, changing quickly to red and gradually paling | bluish violet, then brown |
| Morphine | evanescent blue, then intense blue, green to persistent olive green | brown |
| Narcein | faint greenish yellow, then violet | dark violet |
| Narcotine | greenish steel blue, later cherry red | |
| Nicotine | yellowish | yellowish |
| Papaverin | greenish, dark steel blue, then deep violet resembling methyl violet | intense dark methyl violet |
| Physostigmine | brownish yellow | weakly brown |
| Picrotoxin | almost colorless | yellowish brown |
| Quinine | colorless | dark brown |
| Solanin | gray-brown | gray-brown |
| Strychnine | colorless | colorless |
| Thebain | deep orange, gradually paling | dark brown |
| Veratrin | citron yellow, later olive green | brownish violet |

Table I shows that Mecke's reagent produces characteristic color effects with the opium alkaloids, morphine, codeine, narcein, narcotine, papaverin, thebain and apomorphine. To these we may add dionin or ethyl morphine and heroin or diacetyl morphine, for we have found that these two morphine derivatives yield an evanescent blue, changing quickly to the characteristic emerald green.

In a number of comparative tests Mecke found his reagent more sensitive than many of the older ones. The characteristic reaction appeared immediately in the cold. In practical work, which involved the testing for the presence of alkaloids in tissue, the heat necessary for extraction processes gives rise to brown impurities, which often hide the color effects produced by reagents. He observed, however, that the color which the selenium reagent produced was always noticeable in the brown extraction mixture. According to Morgulis and Levine, 0.01 milligram of morphine, equivalent to 0.0125 milligram of morphine sulphate, yields a strong positive reaction, while 0.005 milligram of the alkaloid equivalent to 0.0125 milligram of the sulphate gives a faint but recognizable reaction.

Corrected in Dec. no. 0.025 —

The colors observed for brucin, colchicin, delphenin and veratrin are no doubt due to the sulphuric acid in the reagent. Concentrated sulphuric acid alone is said to give yellow, orange or brown colors with veratrin, colchicin, delphenin, gelsemin and lobelin. Veratrin in the presence of concentrated sulphuric acid assumes an intense yellow color and finally dissolves to a yellow solution. This soon changes to an orange shade with a strong greenish fluorescence shown by reflected light. The separate alkaloids of veratrum album and viride show changes in sulphuric acid, varying from red to violet or green.

The Lafon or Mecke reagent is not specific for the alkaloids of the opium group. Table II lists a number of compounds that give a positive reaction. The reagent employed consists of 0.5 per cent selenium dioxide or 0.75 per cent sodium selenite in pure concentrated sulphuric acid.

As seen from the results, phenolic compounds in contact with a solution of 0.5 per cent selenium dioxide or 0.75 per cent sodium selenite in pure concentrated sulphuric acid yield a pale green, olive green, bluish green, or purplish green color. Sometimes several shades are observed simultaneously. More often one shade gives way to a succession of shades. In addition to the characteristic color produced by the phenol, there is often observed the concomitant decomposition of the selenious-sulphuric acid reagent with the formation of a brick-red or brown colloidal solution, suspension, or precipitate of free selenium. The characteristic coloration produced by the positive-reacting compound is at first so intense that the brown-red or brick-red selenium does not mask it. Many organic compounds, notably carbohydrates, reduce the selenious-sulphuric acid reagent with the formation of free red selenium. The characteristic colors formed in the presence of phenols, however, are not permanent. They disappear on standing and are subsequently replaced by the red or brown selenium tint. The direct addition of water to the greenish reaction mixture results in the rapid fading of the green and the appearance of a reddish or brownish coloration.

The characteristic color reaction may be explained on the basis of the fact

TABLE II

| | |
|---|---|
| **MONOPHENOLS** | |
| Phenol | Grass green. The intensity of the color begins to diminish after 2½ hours. |
| o-Cresol (methyl phenol) | Dark green, fading in about 1 minute to brown with a pink tinge. |
| m-Cresol | Deep emerald green. The color is more intense after 1 hour. Very little of the original color remains after 2 hours. |
| p-Cresol | Fleeting olive green, which can be noticed about the edges of the container, finally changing to brown. |
| o-Xylenol (dimethyl phenol) | Reddish brown, changing in about 1½ minutes to reddish purple, and in 2 minutes to deeper red shade. |
| m-Xylenol | Deep red, changing to reddish brown in 1 hour. |
| p-Xylenol | Purplish, changing to characteristic green and fading in about 12 minutes. |
| Thymol | A beautiful play of colors. Yellowish, bluish, purplish, cherry red and bright green colorations are observed in succession or simultaneously. The color display is very distinct at the sides of the crucible. |
| Carvacrol | Bright green on sides and bottom of crucible. Reddish brown also appears simultaneously with the green. The coloration persists 3 hours, especially along sides of crucible. |
| α-Naphthol | Deep emerald green, giving way in 20 minutes to muddy rust brown precipitate. |
| β-Naphthol | Very slight indication of fleeting olive green, which is replaced by brown. |
| **MONOPHENOLS WITH ALCOHOL GROUP** | |
| Diathesin (o-hydroxybenzyl alcohol) | Purplish to cherry red in sulphuric acid alone; dark brown in selenious-sulphuric acid. |
| **MONOPHENOLS WITH ALDEHYDE GROUP** | |
| Salicylic aldehyde (o-hydroxybenzaldehyde) | Yellowish green giving way to crimson. The greenish tinge disappears after 1 hour. |
| **MONOPHENOLS WITH CARBOXYL GROUP** | |
| Salicylic acid (o-hydroxybenzoic acid) | Very faint trace of green which disappears immediately. |
| Aspirin (acetyl salicylic acid) | No color changes in the reaction mixture. |
| Tyrosine | Brown and olive green, giving way to a bluish green and then to an intense blue, finally disappearing after 5 hours. |
| β-oxynaphthoic acid | Dark brown with tinge of olive green and greenish blue, which persists after three hours. |
| **ESTERS AND SALTS OF PHENOLIC ACIDS** | |
| Oil of wintergreen (methyl salicylate) | Fleeting light green. Very faint green may still be observed after 1 hour. |
| Salol (phenyl salicylate) | Light yellowish green, changing to grass green in 1 minute, later to characteristic bluish green. This color fades in about 45 minutes, giving way to pink. |
| Sodium salicylate | No change in reaction mixture. |
| **MONOHYDRIC PHENOLS WITH HALOGEN** | |
| Di-iodothymol | No change. |
| **NITRATED MONOHYDRIC PHENOLS** | |
| o-Nitrophenol | No change. |
| p-Nitrophenol | No change. |
| Dinitrophenol | No change. |
| Picric acid | No change. |
| **MONOHYDRIC PHENOLS WITH AMINO GROUP** | |
| p-Amidophenol hydrochloride | Purple color with a slight greenish tinge. With sulphuric acid alone a bluish tinge, which disappears almost immediately, giving place to a black color. |
| Photol (mono-methyl p-amidophenol hydrochloride) | Turns pink in 2 minutes; changes to reddish brown with slight purple tinge in 4 minutes. |
| Metol (methyl-amino-cresol sulphate) | Faint pink appearing after ½ minute; changes to cherry red. With sulphuric acid alone no characteristic reaction. |
| Amidol (2, 4 diamino phenol sulphate) | Dark purple changing to blue, and finally changing to red within 18 hours. With sulphuric acid alone a bluish color develops. |
| **MONOHYDRIC PHENOLS WITH NITRO AND AMINO GROUPS** | |
| Picramic acid (amino-dinitro phenol) | No reaction. |

TABLE II—CONT'D

| | |
|---|---|
| **ETHERS OF MONOHYDRIC PHENOLS** | |
| Anisole (methyl phenyl ether) | Dark green persisting after 3 hours. |
| Phenetole (ethyl phenyl ether) | Emerald green at bottom of crucible. Surrounding liquid is pink. After an hour the green is still to be observed, but begins to fade after 3 hours. |
| **ETHERS OF MONOHYDRIC PHENOLIC ACIDS** | |
| Anisic acid (p-methoxy benzoic acid) | Grass green giving way to brown coloration in about 2 hours. |
| **DIHYDRIC PHENOLS** | |
| Pyrocatechin (o-dihydroxybenzene) | Beautiful emerald green, persisting after 18 hours. This color gives way to an intense blue on the second day and to a blue precipitate on the third day. |
| Adrenalin (o-dihydroxyphenol hydroxy-ethyl methylamine) | Emerald green, giving way within 10 minutes to red brown. |
| Resorcin (m-dihydroxybenzene) | Yellowish brown, giving way to red brown in one hour. |
| Orcinol (methyl resorcinol) | Grass green, changing to deep olive green almost immediately. The color persists but takes a slight brownish tinge in 15 minutes. A dark brown remains to be seen at end of 45 minutes with only a slight tinge of green. |
| Hydroquinone (p-dihydroxybenzene) | Brown and purplish coloration with a tinge of green. A slight greenish tinge persists in the purplish brown after 1 hour. |
| **ETHERS OF DIPHENOLS** | |
| Guaiacol (monomethyl ether of pyrocatechol) | Dark olive green persisting after 2½ hours. |
| Eugenol (allyl 4, 3 guaiacol) | Yellowish green, giving way to crimson after 1 hour. The sides of the crucible show a purplish coloration. |
| **ETHERS OF DIPHENOLS WITH ALDEHYDE GROUP** | |
| Vanillin (m-methozy-p-hydroxy benzaldehyde) | Yellowish green. Slight trace of green is still present after 1 hour. |
| Piperonal (heliotropine) (anhydride of vanillin) | Orange and bright green. After 1 hour the green increases in intensity. |
| **ETHERS OF DIPHENOLS WITH CARBOXYL GROUP** | |
| Vanillic acid (m-methoxy p-hydroxy benzoic acid) | Slight tinge of yellowish green, fading completely after 1 hour. |
| **TRIHYDRIC PHENOLS** | |
| Pyrogallol (1, 2, 3 trihydroxy benzene) | Brownish black color, becoming almost black in 5 minutes. |
| Phloroglucin (1, 3, 5 trihydroxy benzene) | Beautiful emerald green, fading within 30 minutes. |
| **TRIHYDRIC PHENOLS WITH CARBOXYL GROUP** | |
| Gallic acid (3, 4, 5 trihydroxybenzoic acid) | Beautiful emerald green persisting after 10 minutes. |
| Tannic acid (digallic acid) | Olive green tinge at first, then to dark brown. At end of 24 hours the reaction mixture is reddish. |
| Dermatol (basic bismuth gallate) | Dark olive green changing to blue in 3 to 4 minutes, and changing back to green after 20 minutes. |
| **GLUCOSIDES YIELDING PHENOLS ON HYDROLYSIS** | |
| Arbutin (glucose + hydroquinone) | Yellowish brown changing to dark brown in 1 minute. |
| Esculin (glucose + 4, 5 dihydroxy coumarin) | Yellowish green disappearing in 1 hour. |
| Phloridzin (glucose + phloretic acid + phloroglucin) | Olive green, becoming deep bluish green and finally giving way to a red brown precipitate. |
| Salicin (glucose + o-hydroxy benzyl alcohol) | Beautiful cherry red. The same color reaction takes place with sulphuric acid alone. |
| Saponin | Light green changing immediately to brown. Slight green color at edges, which persists for 1 hour. |

that selenious acid is a compound which is readily reduced, yielding oxygen. The phenols reduce the selenious acid and they in turn undergo oxidation with the formation of chromogenic derivatives. The appearance of the red or brown-red color after the primary color reaction is due to the selenium released in the course of the decomposition of the selenious acid. The view that the green color is caused by the free selenium dissolving in the concentrated sulphuric acid to form a green solution of selenosulphur trioxide ($SeSO_3$) is untenable, since lactic acid, glucose and other carbohydrates react with the selenious-sulphuric acid to give only a red or brick-red coloration characteristic of elemental selenium.

From the fact that the selenium reagent gives a positive reaction with phenols, it seems plausible to postulate that the phenolic hydroxyl in the morphine alkaloids is responsible for the reaction described by Lafon, da Silva, Mecke, and others. Morphine contains two hydroxyl groups, one of which is phenolic. Codeine is a phenolic ether, methyl morphine. Dionin is ethyl morphine; heroin, diacetyl morphine. Apomorphine is made from morphine by the removal of a molecule of water as a result of dehydration with concentrated hydrochloric acid. Thebain has two methoxy groups, narcotine and narcein three, and papaverin four. The characteristic reaction is given by free phenols as well as those in which the hydrogen of the phenolic hydroxyl is substituted by an alkyl radical. In this respect the reagent differs from ferric chloride, which does not react with phenolic ethers like codeine, anethol and phenetol. The sulphuric acid of the selenium reagent decomposes the methoxy group with the liberation of the free phenol.

Besides the phenolic alkaloids of the opium group, berberine also yields a distinct reaction. Ferreira da Silva observed that it gave a yellowish green, which turned to brown, then to rose at the edge of the reaction mixture and violet in the middle. Berberin contains two methoxy groups. Quinine has one hydroxy and one methoxy group, but it does not give the phenol reaction. The neighboring groups in the molecule probably interfere with the reaction as they do in the case of some of the nonreacting phenols.

The selenious-sulphuric acid reaction which we propose as a specific reaction for the phenol group is one of wide applicability. This reaction is positive with monophenols and monophenolic ethers, or monophenols with such substituents as halogen, amino group, aldehyde or carboxyl group, with diphenols and their derivatives, with triphenols, with phenolic glucosides, and phenolic alkaloids. The reaction besides enjoying wide applicability is also very delicate. Only a very minute quantity of the solid substance, even a fraction of one crystal, is necessary for a positive response. A small quantity of dry material, preferably in powdered form, is placed in a small white porcelain crucible and the selenious-sulphuric acid is allowed to flow drop by drop down the sides of the crucible. About five drops are added. Usually the first drop making contact with but a minute quantity of the substance in the crucible brings out very vividly and almost instantaneously the characteristic green coloration. It is advisable to rotate the crucible sideways, as very often a play of colors is observed at the edge of the mixture. If the phenol is dissolved in water or in an organic solvent it may first be evaporated to dryness on the water-bath. A drop or two

of sulphuric acid may be added during the evaporation to prevent any rapid oxidation of the phenol.

The test proposed also lends itself to a beautiful zone reaction. The phenol may be dissolved in an organic solvent, which in itself does not decompose the selenious-sulphuric reagent with the liberation of selenium, or the aqueous solution of the phenol may be extracted from such solvent. Methyl alcohol, ethyl alcohol, ether and caprylic alcohol are not available because of the decomposition they induce in the selenious-sulphuric acid mixture. Amyl alcohol brings about very slight decomposition and chloroform none whatever. Both of these compounds are excellent solvents for morphine. The addition of selenious-sulphuric acid to an amyl alcohol or, preferably, chloroform solution of a phenol results in the formation of a beautiful emerald green ring at the point of junction of the two immiscible liquids. The green zone lies as a colored layer on the surface of the sulphuric acid and finally gives way to solid green particles, which float at the juncture, slightly submerged under the selenious-sulphuric acid.

The color reaction is sometimes influenced in its course by the substituent groups on the benzene ring carrying the phenolic hydroxyl. Nitrating the phenol abolishes the reaction, for o-nitrophenol, p-nitrophenol, dinitrophenol, trinitrophenol (picric acid) and amino dinitrophenol (picramic acid) fail to respond to the test. The presence in the molecule of nitro groups, derived from such a strong oxidizer as nitric acid, exerts a retarding influence upon the reducing capacity of the phenol. The presence of an aldehyde or carboxyl group on the benzene nucleus also inhibits the reaction, for salicylic aldehyde, salicylic acid, vanillin and vanillic acid yield exceedingly faint responses. Acetyl salicylic acid proves entirely negative. Esters of salicylic acid, however, react strongly. While thymol responds in a most vigorous way, di-iodothymol has no effect on the reagent.

Of all the phenols, pyrocatechol gives the most durable and the most vivid color. In testing for selenium in impure sulphuric acid it would be advisable to employ pyrocatechol instead of codeine phosphate, recommended by E. Schmidt and also by Schlagdenhaufen and Pagel.

The color reaction also admits of the differentiation of $\alpha$-naphthol from $\beta$-naphthol. The former yields a deep emerald green, persisting for twenty minutes, while the latter gives a very slight indication of a fleeting olive green. The selenious-sulphuric acid reagent is also useful in distinguishing acetic anhydride from glacial acetic acid. The addition of the anhydride to the reagent results in the formation of elemental selenium, which appears as a brick-red collodial solution or precipitate. Glacial acetic acid, however, is not affected by the reagent.

## CONCLUSIONS

1. Selenious acid (or its alkali salts) dissolved in pure concentrated sulphuric acid forms a colorless reagent yielding a characteristic color reaction specific for the phenol group.

2. The color test is extremely sensitive and possesses wide applicability. It is given by monophenols, monophenolic ethers, monophenols with halogen,

amino, alcohol, aldehyde or carboxyl group, diphenols and their derivatives like ethers, etc., triphenols, phenolic glucosides, and phenolic alkaloids.

3. The intensity of the reaction is modified by the presence of various substituents besides the phenolic hydroxyl in the benzene nucleus. The presence of one or more nitro groups interferes with the reaction completely.

4. The selenious-sulphuric acid reagent of Mecke, Lafon and others gives the reaction characteristic of the morphine alkaloids because of the presence of the free phenolic hydroxyl or of one liberated by the interaction of sulphuric acid with one or more methoxy groups present.

5. Mecke or Lafon's test is not distinctive of the morphine alkaloids. In toxicologic examination of tissue it must be kept in mind that phenol, p-cresol, tyrosine, pyrocatechin, hydroquinine and adrenalin are phenolic compounds of biologic occurrence. A positive test is of no absolute significance, but a negative test is important as a conclusive indication of the absence of the morphine alkaloids.

6. The selenious-sulphuric acid reagent also serves to differentiate $\alpha$-naphthol from $\beta$-naphthol, and glacial acetic acid from acetic anhydride.

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# THE EFFECT OF ATROPINE AND THE RÔLE OF THE INVOLUNTARY NERVOUS SYSTEM IN INSULIN ACTION*

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FORMER studies[1, 3] of the authors, which are published in several articles, have dealt with the effect of insulin after different methods of administration. It has been found that there is a striking difference between the effect of insulin when like amounts are injected intradermally, subdermally or intravenously. The effect of intravenous injection lasts only for a short time and it has been known since the first publications of Banting and Best[2] that the subcutaneous administration is even more effective than the intravenous method, and, consequently, it has since been the method of choice in diabetic treatment.

Our work was started with the idea of finding more definite data about the clinical action of insulin, based on the knowledge that differences in the effect of the drug manifest themselves when different body tissues are used for injection. Though it may be stated that an injection of a nonspecific albumin solution, such as Aolan, acts more intensively if it is made into the skin than if it is injected subdermally, yet, if the dosage of such nonspecific agents is diminished to very small amounts, a stage is reached in which only injections within the skin are effective, while subdermal and intravenous injections of equal amounts do not show any measurable effect.

This effect of nonspecific agents can be measured by the effect on the involuntary nervous system, more particularly on its parasympathetic part, stimulation of which leads to a sudden displacement of the leucocytes in human beings. This displacement is noticeable in a peripheric leucopenia of a short duration, a symptom of which cannot be shown in the animal body because of the differences in the skin of animals and human beings.

With this knowledge in mind, we thought first that different kinds of injection of insulin with their different effects might lead to a more intimate knowledge of insulin action. Furthermore, we believed that the same explanation as mentioned above, which has shown the close relationship of the skin to the involuntary nervous system, might lead to the discovery of new facts concerning the action of insulin.

In our first publications we have demonstrated that like amounts of insulin have a larger effect if administered intradermally than if administered subcutaneously. These findings have been obtained from differences in the blood-sugar lowering effect of insulin in a series of animals which were used, after a short period of rest, for the different methods of administration of insulin, so that we could base our results upon the difference in different animals as well as in the same individual.

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How this difference occurred has not yet been explained. It will be the subject of this paper to describe further studies made for this purpose. As has been shown in the foregoing articles, as far as animals are concerned, the blood-sugar lowering effect of insulin begins almost at the same time and reaches almost similar levels. In some animals a lower level is reached earlier by the intradermal than by the subdermal mode of administration at the two-hour test. After the second hour a very noticeable difference in the blood-sugar curves showed a longer lasting and more intensive effect of the intradermal mode of administration. This may be demonstrated by two comparative curves giving the average of ten curves of the effect of the intradermal and subdermal modes of administration. The animals used were the same in both tests.

In explanation of this difference it seems reasonable to consider the difference of the tissues and other absorption properties as contributing causes to the differences in insulin action. We know that resorption from the skin takes

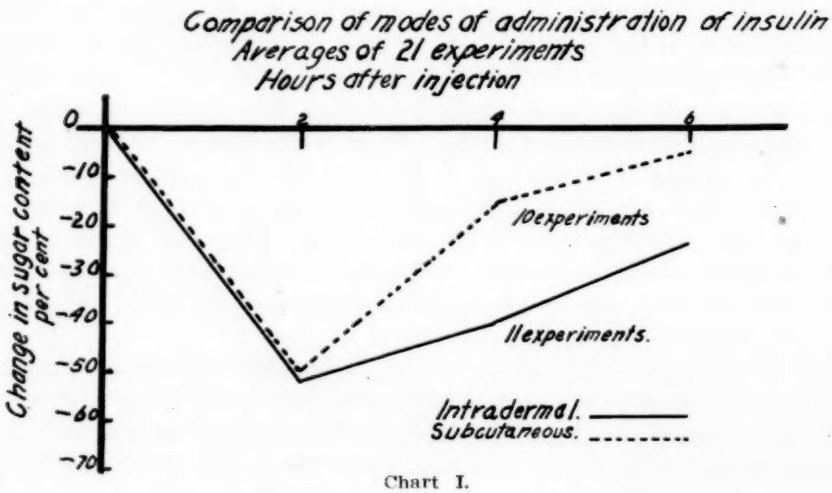


Chart I.

longer than from the subdermal tissues, i.e., insulin or any other substance injected into the skin will be present in the circulation later than after injection of the same into the subdermal tissues. This has been confirmed by the recent paper by Kasahara.[4]

Furthermore, it has to be considered that the longer the route of resorption, the more of the effective part of the absorbed agent will be lost by adsorption. These considerations show that in our experiments, insulin injected into the skin must reach the circulation later and in smaller quantities than the subdermally and the intramuscularly injected dose.

Moreover, we have to consider that during a given period smaller amounts of insulin reach the circulation from the skin than from a like injection into the subdermal tissues, thus showing that during this time, for instance, over a period of two hours after the injection has been performed, a smaller amount of insulin must be present in the circulatory system from the intradermal insulin deposit than from a like subdermal deposit.

All this does not agree with the observation that the measurable effect of

insulin after two hours is the same after subdermal and intradermal injection. Furthermore, it cannot be understood that, with definitely smaller amounts present in the circulation, the same or a lower blood-sugar content is really obtained, if one considers insulin active only after entering the blood stream. Experience shows that the effect in the circulation depends closely upon the dosage present. Thus small amounts injected intradermally must have a small effect and large doses a greater effect.

We have then, in brief, this condition: With the intradermal injection a larger amount of sugar is metabolized than after a subcutaneous injection of a like quantity of insulin. Yet, at any given time, there is, in all probability, a lower concentration of insulin in the circulatory system after intradermal than after subdermal injection. This leads us to believe that from our experiments we cannot conclude that the actual blood-sugar lowering effect depends entirely upon the amount of insulin present and active in the circulation. On the contrary, we have to assume that there must be some other way of insulin action in addition to the distribution of the drug by the circulation. This leads to the only possible conclusion that a postponed absorption produces a larger effect than an accelerated entrance into the circulation by subcutaneous administration. Thus the previously described findings of our experimental work in rabbits indicate that there must be another route of insulin action than the well-known effect by distributing the drug throughout the circulation which then acts in connection with the body tissues (muscles and liver) in reducing the blood-sugar level.

The question then arises as to how this other action is possible. We have mentioned in the beginning of this paper that from research on the action of nonspecific agents which has been confirmed by many authors, it has been found that the skin is closely related to the involuntary nervous system and injections into the skin always show a nonspecific reaction of the involuntary nervous system, consisting of a parasympathetic overbalance of the splanchnic region, while the periphery is overbalanced in the contrary direction. We cannot go into a more detailed description of these findings which may be found in the respective publications, but the striking parallelism between nonspecific agents and their action and insulin and its blood-sugar lowering effect, depending upon whether injected within the skin or under the skin, particularly called our attention to the fact that the involuntary nervous system may play an important part in the action of insulin if injected intradermally. Such an action of the involuntary nervous system may provide the missing link in the explanation proposed above.

We have stated that there must be a route of insulin action other than that by distribution of the hormone by the circulation, and now the more definite question arises: Does the involuntary nervous system represent this missing link, and is it possible to demonstrate this by definite experimental observation?

Two routes were feasible for this work. In former work with nonspecific agents[5] the involuntary nervous system has been interrupted by pharmacodynamic substances as well as by the knife, and in both cases the action was entirely cancelled, showing that the involuntary nervous system represents a "conditio sine qua non" for the nonspecific effect. In the case of insulin and

its action from the skin deposit, the involuntary nervous system may play a similar important part, and like experimental conditions might allow us to prove it. The involuntary nervous system is, in general, divided into two different parts, the sympathetic and the parasympathetic.

If one wished to interrupt the parasympathetic part by pharmacodynamic substances, two methods are feasible. The parasympathetic part can be blocked by increasing the sympathetic tonus by adrenalin, but this method is not suitable for our experiments. As mentioned above this would lead to an immediate increase of the blood-sugar level and thus hide the insulin effect, and one could not determine if the effect is eliminated by interrupting the parasympathetic system or by an overpowering glycemia following the adrenalin injection.

The other possibility is to paralyze the parasympathetic system by atropine. Atropine, if given in doses of 10 mg. per kg. body weight, does not affect the blood sugar. Therefore it was possible to use the blocking action of atropine for our studies. We injected a series of animals with the same amount (0.5 unit) of insulin per kg. body weight employing the intradermal, the subdermal and the intravenous mode of administration in the same animals after a period of rest of at least seven days. Simultaneously with the injection of insulin, atropine was injected subcutaneously, and, in some experiments, this atropine injection was followed by another injection of the same dosage thirty minutes later. Blood-sugar determinations were made at intervals of thirty minutes, and the following results, tabulated below, have been found:

TABLE I

THE EFFECT OF ATROPINE ON THE ACTION OF INSULIN, AS MEASURED BY THE CHANGE IN BLOOD-SUGAR CONTENT (PER CENT)

| RABBIT | INTRAVENOUS | | SUBCUTANEOUS | | INTRADERMAL | |
|---|---|---|---|---|---|---|
| | 30 MIN. | 60 MIN. | 30 MIN. | 60 MIN. | 30 MIN. | 60 MIN. |
| 87 | −59.0 | −44.8 | −36.2* | −56.7 | −15.3* | −45.3 |
| 88 | −54.5 | −43.9 | −53.8* | −59.7 | −21.5* | −35.6 |
| 76 | −58.5 | −52.5 | −44.7* | −65.8 | −18.8* | −58.6 |
| 58 | No experiments | | −45.6 | −49.7 | −27.8 | −47.3 |
| 78 | −49.5 | −22.0 | −33.4* | −47.8 | −10.4* | −32.0 |
| 79 | −47.4 | −10.0 | −47.3 | −49.1 | + 6.9 | +13.8 |
| | −61.9 | −40.5 | | | | |
| 82 | −35.9 | +17.9 | −16.0* | −28.0 | + 4.6* | + 5.3 |
| Average | −52.4 | −36.93 | −39.57 | −50.97 | − 9.25 | −25.4 |

*A second injection of atropine at the end of thirty minutes following the first bleeding.

TABLE II

THE CHANGE IN BLOOD-SUGAR CONTENT AFTER INJECTION OF INSULIN ALONE (PER CENT)

| RABBIT | INTRAVENOUS | | SUBCUTANEOUS | | INTRADERMAL | |
|---|---|---|---|---|---|---|
| | 30 MIN. | 60 MIN. | 30 MIN. | 60 MIN. | 30 MIN. | 60 MIN. |
| 87 | −53.2 | −34.0 | −47.7 | −50.8 | −34.4 | −46.6 |
| 88 | −46.3 | −19.0 | −55.9 | −57.3 | −27.9 | −44.2 |
| 76 | −62.9 | −33.1 | −34.1 | −52.1 | −19.8 | −44.7 |
| 58 | No experiments | | −47.3 | −50.3 | −19.7 | −31.2 |
| 78 | −55.7 | −33.9 | −39.3 | −44.4 | −19.9 | −36.8 |
| | | | | | −26.5 | −48.5 |
| | | | | | − 7.4 | − 8.3 |
| 79 | −47.0 | −0.0 | −24.8 | −40.7 | −12.5 | −40.0 |
| 82 | −12.5 | +23.2 | −30.3 | −26.8 | − 1.6 | + 4.1 |
| Average | −46.2 | −16.1 | −39.91 | −46.06 | −18.5 | −32.9 |

If one compares these findings with the control results without atropine, the difference is striking. With atropine the intradermal injection of insulin is much less powerful than when the same animal is injected with insulin alone.

Furthermore, the effect of the intradermal injection does not reach the same level and is much less effective than after subdermal injection. If one compares both series of subdermal injections, with and without atropine, in some of the animals, atropine has lessened the effect of the drug a little, but in most cases not at all.

Comparing the intravenous injection and its effect, there is no difference whether or not atropine has been added. Thus it is shown that atropine, which does eliminate to a certain degree the action of the parasympathetic nerves

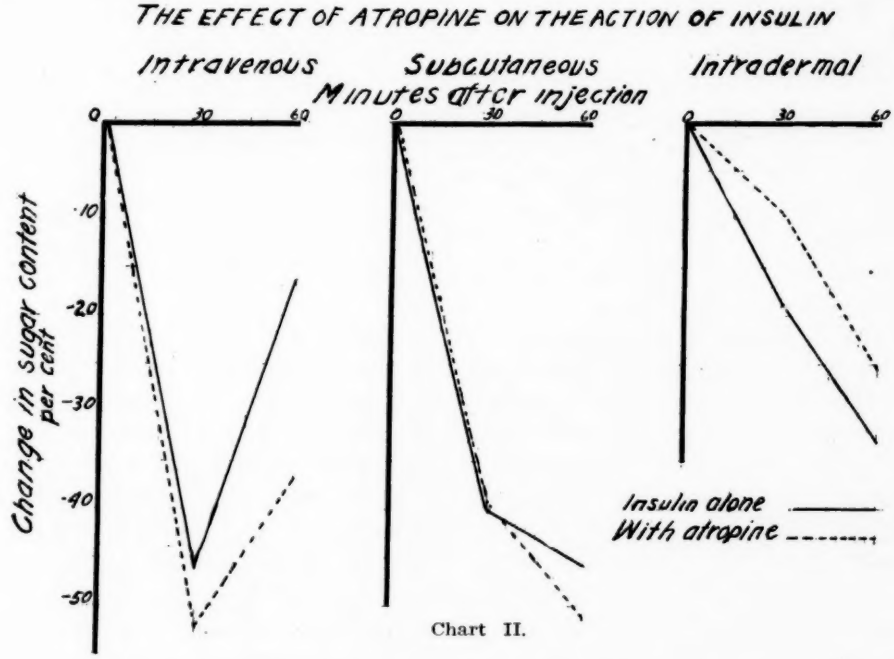


Chart II.

for a certain time, changes the action of insulin according to the method of administration. The intravenous and the subdermal injections of insulin are not affected at all, while the intradermal injection loses one-half of its power. This shows without doubt that atropine, which probably does not undergo any chemical combination with insulin but which without doubt paralyzes the function of the parasympathetic system to a certain degree, has diminished the insulin action. *This diminution is possible only if the paralyzed parasympathetic system has played a part in the insulin action.*

We have previously stated that the direct hormone action within the circulation and within the body fluids cannot be the only basis of the blood-sugar lowering effect. We have stated that there must exist another route of insulin action outside the circulatory system, and this other action must be especially powerful by intradermal injection, less by subdermal, and almost lacking by intravenous administration. The atropine experiments show that by blocking the parasympathetic system, a great deal of the insulin action is lost after intradermal injection, none is lost after subdermal and none after intravenous

administration. *This demonstrates that the missing link in the explanation of the large insulin effect from an intradermal deposit is represented by an action of the involuntary nervous system.* We cannot conclude from these experiments directly in what way the involuntary nervous system is able to produce this blood-sugar lowering effect, but we know, from other experiments in human beings and from the physiologic experience of blood-sugar metabolism, that the liver, to a certain degree, is responsible for the blood-sugar balance by forming glycogen and glucose, respectively. We have learned from experiments initiated by Claude Bernard, that stimulation of the sympathetic system results in a glucose formation. It is further known that the stimulation of the parasympathetic system results in a glycogen formation.

Minkowski's[6] experiments have proved that not only depancreatization but also the severing of the nerves between pancreas, duodenum and liver results in complete diabetes, thus showing how much the nerves participate in the pancreatic and hepatic action. It would take much too long to enter into details provided by the extensive research done on diabetes in almost every country. But from the facts mentioned we know that parasympathetic action leads to an increase of the glycogen formation, and we feel justified in concluding that the definitely proved part that the involuntary nervous system plays in insulin action consists of a nervous stimulation of the glycogenic function of the liver.

This explains how insulin, even if present only in small amounts of the active form in the tissues, may create a pronounced effect upon the blood-sugar level. The stimulus, traveling along parasympathetic fibers from the skin to the liver can result very quickly even though insulin is not present in the blood stream, in a glycogenetic process which takes glucose from the circulation, thus diminishing the blood-sugar level. This also explains how it is possible that the total effect of the same amount of insulin is less after subdermal than after intradermal injection. After subdermal injection the drug enters the circulation comparatively quickly and acts there as a hormone. It loses its power by being eliminated from the blood stream. In intravenous injection the action depends upon the dosage, i.e., upon the actual amount of insulin present. From the combined experiments with intravenous insulin injection and atropine administration we know that practically no neural effect exists if insulin is present only in the blood stream.

We are not able to say to what extent the subdermal tissues provide a neural stimulus as compared with the skin; but we know that the nerve effect from the skin is the most intensive, and, as the deposit is being diminished, the nerve action is lost. (We have to consider that it is impossible to eliminate the entire nerve action. If this were possible it would probably result in a complete loss of all insulin effect so long as only a deposit existed in the skin and no insulin were absorbed into the circulation.) This would account for the early onset of the actual blood-sugar lowering effect from the skin deposit. It also accounts for the more lasting effect and explains the reason for such a large total effect after intradermal injection. Small amounts of insulin will continue to be absorbed from the intradermal deposit after the entire amount of insulin, which has been injected subcutaneously, has been exhausted. Smaller

amounts may still enter the blood stream from the intradermal deposit, while the above-mentioned nerve effect is still present. This makes it possible that, even after the exhaustion of the subdermally- and intravenously-given insulin, the blood-sugar reducing effect may bring the total amount of actually metabolized sugar to a higher degree than any other kind of insulin administration.

It may be mentioned in this paper that experiments in human beings with the same object in view have shown similar differences following intradermal and subdermal injection, thus confirming these findings.[7]

Many other theoretic conclusions may be drawn from the above statements, and, in connection with the many publications on insulin, it may be possible now to explain several observations on the basis of this nerve effect. Magenta and Biazotti[8] first showed that atropine has an antagonistic action to insulin. Many other authors point to similar ideas. Giving more details would lead beyond the limits of our paper. We merely wanted to show that many previous publications have pointed in the same general direction, but we believe that the difficulty in determining more exact data has been that the entire previous work was based only upon one kind of insulin administration, namely, the subdermal injection. The observed differences between the intradermal and the subdermal administration gave us the clue to develop the reasons for this difference.

## CONCLUSIONS

Our conclusions are: A deposit of insulin in the body acts by a nerve stimulation, very probably of the glycogen-forming function of the liver. This nerve stimulation is conducted by way of parasympathetic fibers. The effect is active as long as the insulin deposit exists. It is stronger if the insulin deposit is made in an organ which has an especially close relationship to the involuntary nervous system, as has the skin, and if the absorption is very slow. It is weaker after deposition of the insulin in organs where absorption takes place rapidly, for instance, the subdermal tissues, and is lacking after intravenous administration. The neural effect of insulin does not depend upon the dosage of the deposit, while the hormone action in the circulation and in the body fluids does depend upon the dose. Simultaneously with the beginning of the absorption from the insulin deposit and with the entrance of insulin into the circulation, the direct hormone effect is manifested. With this hormone effect and the conclusive destruction of the hormone and the elimination by natural channels, the nerve effect decreases, together with the diminution of the insulin deposit outside of the circulation, and disappears as the last part of the insulin deposit is absorbed.

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# THE EFFECT OF ORALLY ADMINISTERED MUCIC ACID UPON RENAL FUNCTION*

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In a series of investigations on the relation of chemical structure to the nephropathic action of the dicarboxylic acids we[1] have shown that mucic acid, when administered subcutaneously as its sodium salt, is extremely destructive to the kidneys. The injection of 0.25 gm. to 0.50 gm. in rabbits induces a severe nephritis of the tubular type, accompanied by an almost complete or total loss of the power of phenolsulphonephthalein elimination, and an enormous retention of nitrogenous waste products in the blood. In some cases anuria occurs, and persists for a period of one to two days following the acid administration.

In the course of our discussion of these results, we pointed out that the data may have a practical value, inasmuch as it is reported that mucic acid is now being manufactured on a large scale with a view to its use as a component of baking powders and other food products. Obviously, the fact that mucic acid exerts a deleterious action when introduced parenterally, does not justify one in assuming that the same effect would follow its oral administration. It is quite conceivable that this substance, like some other dicarboxylic acids when given per os, may be rejected by the alimentary cells, and thus fail to come in contact with the kidneys. Furthermore, should the acid be absorbed, there is the additional possibility that it might be detoxicated by the organism, and in this fashion be prevented from exerting injurious renal effects. With these considerations in mind we have conducted a series of experiments involving the feeding of mucic acid.

Rabbits were employed as the experimental animals. In all cases food was withheld, but water was supplied *ad libitum*. This procedure was followed in order to insure constant conditions throughout the experiments, inasmuch as animals refuse to eat after the development of severe nephritis. Furthermore, Salant and his associates[2] have shown that certain foods render rabbits less susceptible to the action of tartaric acid. It seemed safer, therefore, to eliminate the possibility of dietary influences by using the animals in a fasting condition. Results so obtained doubtless may be regarded as maximum effects.

As criteria of renal function, we have determined at frequent intervals the quantities of nonprotein nitrogen, creatinine, and chlorides in the blood. After control periods of approximately forty-eight hours, during which time one or two blood samples were removed, the animals received the mucic acid by stomach sound either in the free state, or after neutralization with sodium carbonate. The administration of the sodium salt almost invariably evoked a marked diarrhea, probably due to the liberation in the stomach of relatively

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large amounts of sodium chloride. For this reason, in most of the experiments, the acid was suspended in water and given in the free state. For details as to the methods of analysis, the reader is referred to a former communication.[3]

Twelve experiments were made upon twelve different animals. The doses of mucic acid varied from 1 to 19 gm. With the smaller doses (1 to 5 gm.) no effects upon renal activity were observed. With the larger doses (5 to 19 gm.) nephritis was occasionally seen. Indeed, four of the twelve animals manifested well-defined cases of tubular involvement. The results of two positive experiments are shown in Tables I and II. In the first, two 5 gm. doses of mucic acid were administered on succeeding days. As will be observed, the nonprotein nitrogen of the blood increased from approximately 42 mg. per hundred cubic centimeters (a normal value for rabbits) to 74 mg. per hundred cubic centimeters. The blood creatinine rose from 1.7 to 4.4 mg. per hundred cubic centimeters, while the chlorides remained practically constant.

In Table II are presented the results of an experiment in which 19 gm. of mucic acid were given during a single day. The data indicate enormous increases in both nonprotein nitrogen and creatinine. On the fourth day following the acid administration, the nonprotein nitrogen amounted to 228 mg. and the creatinine to 17 mg. per hundred cubic centimeters of blood. These are the most pronounced retentions we have observed in any of the animals following the oral administration of mucic acid. In a third experiment, the

TABLE I

RABBIT NO. 2, 2230 GM.

| DATE | MUCIC ACID FED. | BLOOD ANALYSIS | | | NOTES |
|---|---|---|---|---|---|
| | | N. P. N. | PREF. CREATININE | NaCl | |
| Nov. | gm. | mg. | mg. | % | |
| 19 | | | | | No food; water ad lib. |
| 20 | | 42.1 | 1.7 | — | 8:30 A.M.—10 c.c. blood removed. |
| 21 | | 42.3 | 1.7 | 0.51 | 8:30 A.M.—10 c.c. blood removed. |
| 21 | 5.0 | | | | 11:00 A.M.—Acid given as sodium salt. |
| 21 | | 43.5 | 2.0 | 0.51 | 8:30 P.M.— 9 c.c. blood removed. |
| 22 | | 62.5 | 3.4 | 0.55 | 1:30 P.M.—10 c.c. blood removed. |
| 22 | 5.0 | | | | 2:30 P.M.—Acid given as sodium salt. |
| 22 | | 74.3 | 4.4 | 0.52 | 10:30 P.M.—10 c.c. blood removed. Animal was unable to stand, and was chloroformed. |

TABLE II

RABBIT NO. 4, 2570 GM.

| DATE | MUCIC ACID FED. | BLOOD ANALYSIS | | | NOTES |
|---|---|---|---|---|---|
| | | N. P. N. | PREF. CREATININE | NaCl | |
| Jan. | gm. | mg. | mg. | % | |
| 9 | | | | | No food; water ad lib. |
| 10 | | 42.6 | 1.7 | 0.51 | 8:15 A.M.—10 c.c. blood removed. |
| 11 | | 41.7 | 1.7 | 0.51 | 8:15 A.M.—10 c.c. blood removed. |
| 11 | 19.0 | | | | Free acid given in two equal doses at 10:50 A.M. and 1:30 P.M. |
| 12 | | 58.8 | 2.8 | 0.54 | 8:30 A.M.—10 c.c. blood removed. |
| 13 | | 151.6 | 8.7 | 0.52 | 8:30 A.M.—10 c.c. blood removed. |
| 14 | | 219.6 | 14.0 | 0.54 | 9:30 A.M.—10 c.c. blood removed. |
| 15 | | 228.8 | 17.0 | 0.56 | 9:00 A.M.—10 c.c. blood removed. Experiment discontinued. |

results of which are not shown in detail, the feeding of 15 gm. of the acid led to values for nonprotein nitrogen of approximately 200 mg.

It is evident from the above data that in rabbits the administration of mucic acid by the alimentary route *may* cause nephritis, but that the dose necessary for such an effect is enormously larger than that which destroys kidney function when the substance is injected subcutaneously. Similar results in the case of tartaric acid have been observed by others. Pearce and Ringer[4] obtained indications of nephritis in one of the three animals in which tartrates were administered per os. Post[5] was unable to observe in man any detrimental effects upon the kidneys following the administration of medicinal doses of Rochelle salts. Apparently mucic acid, like tartaric acid, is largely unabsorbed from the alimentary tract. Only overwhelming doses, such as were administered in some of our experiments, lead to renal involvement in normal animals.

The above data may be interpreted by some as indicating the harmlessness of mucic acid in the quantities which would be present in baking powders. On the other hand, we believe that such an interpretation would be unfortunate. Although the doses of mucic acid employed in our experiments are infinitely larger than could be obtained in man from the quantities of baking powders ordinarily ingested, it is not impossible that the habitual ingestion over long periods of time of minute traces of the substance *might* lead eventually to renal effects in some individuals. Furthermore, we know comparatively little concerning the mechanism of alimentary absorption, and still less regarding variations which may occur in the permeability of the alimentary cells under abnormal conditions. While the "selectivity" of the cells might effectually prevent mucic acid absorption under favorable circumstances, who knows but that increased permeability in abnormal conditions might permit the passage into the circulation of injurious traces of the substance? Inasmuch as nontoxic acid compounds are available for use in baking powders, it would not appear rational to employ a violently nephropathic agent, and depend upon its rejection by the gastrointestinal cells. Therefore, we seriously question the advisability of the use of mucic acid as a component of the human dietary.

## SUMMARY

1. Of twelve rabbits which received doses of 1 to 19 gm. of mucic acid by mouth, four showed distinct evidence of renal involvement, as indicated by marked retention of nitrogenous waste products in the blood.

2. These results would seem to indicate that mucic acid is scarcely a safe substance to be employed as a component of human food products.

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# THE VALUE AND CHARACTER OF REPORTS AND RECORDS OF TISSUE EXAMINATIONS*

By L. A. Turley, A.M., Ph.D., Oklahoma City, Okla.

THE value of tissue examinations is so well recognized that we need waste little time discussing this point. The question before us is the nature of the report and the manner of keeping the records of these tissue examinations. At the meeting of this Society a year ago, the statement was made in an informal lecture that all the surgeon wanted to know was whether or not the tissue examined revealed malignancy. This statement was a surprise to me. I can scarcely believe this is true, but, if it is, it is squarely up to us, as pathologists, to educate the surgeons to a value of tissue examinations beyond this point. Any surgeon who is satisfied with the meager report of "positive" or "negative" in regard to the question of malignancy is fair neither to himself nor to his patients, because he is not availing himself of the information as to the character of the lesion with which he is dealing, and, consequently, he is robbing the patient of the proper treatment. As an example of this, I might briefly recite a recent case in our hospital. The patient came in with symptoms of intestinal obstruction accompanied by loss of weight. Physical examination revealed a mass in the right flank. A diagnosis of malignancy was made from the findings and operative procedures were carried out on the basis of this diagnosis. The postoperative gross diagnosis confirmed the preoperative diagnosis, but the microscopic examination showed the lesion to be tuberculous, which, of course, entirely changed the prognosis and after-treatment of the case. If the surgeon had proceeded with a simple negative report of nonmalignancy and had been satisfied with this report, he would have been utterly in the dark as to the condition with which he was dealing.

There is no laboratory report carried on at the present time which simply indicates positive or negative in regard to any one specific question where any other report is possible. Blood counts are not reported on the basis of positive or negative leucocytosis or erythrocytosis, but a detailed report is made of the exact number, not only of any individual hemocyte but of all of them. In urine examinations, physicians are no longer satisfied with positive or negative reports of the various substances which might be encountered; but, if positive, they desire to know the degree of excess. Similarly in blood chemical procedures, not only a knowledge of the presence of the normal and abnormal but the amount of normal and abnormal constituents is desired. So that we may sum up the proposition as to the character of the report of the laboratory procedure by saying that laboratory reports should be both qualitative and quantitative, for often the amount of abnormality is as important to know as

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the presence of the abnormality. The same thing applies to tissue reports. As was stated before this body last year and as has recently been stated by Ewing, even in cases of positive malignancy, not only the fact of malignancy, but the character of the malignancy should be reported, for it is well recognized that different malignancies differ in degree and habits so that not only immediate treatment but after-treatment and prognosis depend on knowing the character of the malignancy. And, further, tissue examination, whether malignant or not, should be reported in detail. In case of inflammations, the definite type of inflammatory process should be reported, as acute, chronic, proliferative, destructive, catarrhal, diphtheritic. Nor should we stop with some one word, such as those enumerated above, which might characterize the particular inflammation. The pathologist should record in detail all pathologic conditions found, and these should be described in such a way that the diagnosis is apparent to any one who understands pathology at all. In this way and in this way only is the clinician furnished with valuable information for the treating of the present lesion, and also with data by the comparison of which, in a series of cases with the clinical findings, he is enabled in the future to make more accurate diagnoses and differentiations. From my experiences in examining tissues, there has arisen in my mind the question, "Is it not true that appendicitis and salpingitis are confused, because the examination of the salpinx, which was the primary subject of operative procedure, often shows far less disease than the appendix, which was removed as a matter of routine during the operation?" There are also cases in which appendicitis and gastric ulcers have been confused, and it is only by means of the reports of the pathologist that the clinician is furnished with the data necessary to distinguish between these pathologic conditions. Therefore, if the clinical pathologist, in the examination of tissues, wishes to render the service of which he is capable, to the clinician and to the patients, it is necessary that he go into considerable detail, describing the pathologic conditions of all of the tissues in the particular specimens submitted and including the findings in his report to the clinician.

We now come to the consideration of the character of the record of tissue examinations which should be kept. Let us, in this consideration, divide the record into two classes: material and documentary. The material records consist of the slide or slides from which the examination was made. This necessitates the use of a technic which would produce a permanent mount in preparing the slides for diagnosis. In general routine tissue examinations, the time element of the diagnosis is not so imperative but that some of the slower procedures which develop permanent mounts may be used. In suspicious cases, the quicker frozen method, of course, must be employed. But in these cases, if the rapid technic does not give a permanent mount, a permanent mount should be made, as nearly as possible, from the same site as the rapid preparation. The slide, once made, should be carefully labeled and filed. Slide cabinets, with a capacity for thousands of slides, take up very little room. The preservation of the slide is necesary for many reasons: The progress of the case may take such a turn that the clinician would like a review of the examination. On occasion, the clinician may desire confirmation of the opinion of the pathologist by some other pathologist, a request with which, of course, he should

freely and cheerfully comply. The discrepancies in diagnosis, which frequently occur in such cases, are often due to the fact that the two men are examining different slides. In a bone tumor recently submitted to me, three different diagnoses could have been made, each equally correct, depending on the portion of the lesion from which the slide was made. Further, the diagnosis may be questioned and the pathologist himself is protected by having on hand the slide from which he made his diagnosis. Such slides are also valuable as subjects of study in case of research, and, for other minor reasons, the slides which have been examined should be carefully preserved.

The other type of record mentioned above is called documentary for the reason that it is written out and should consist of a report either made to the clinician or placed on the chart of the patient for consideration by the clinician. This report should include a careful and detailed description of the microscopic examination including all pathologic conditions found on examination. It should include the details which would, for example, differentiate the diagnosis of scirrhous carcinoma from medullary carcinoma; the size and general condition of the fibroblasts in a spindle-cell sarcoma, the proportion and distribution of stroma in regard to the tumor cells, etc. In the prostate gland, for example, the atrophy or hypertrophy of the tissues composing the organ; the increase or decrease in stroma; the amount, character, and distribution of infiltrating cells, the presence or absence of edema or congestion; the condition of blood vessel walls, etc., all should be carefully made out and recorded. When such a description is complete, the clinician will be able to know the exact state and condition of the afflicted organ, and the diagnosis will be evident.

In our own hospital we proceed as follows: First, there is a description made of the gross material brought from the operating room or the autopsy table, and in all cases this should be a description, not a diagnosis. For example, "blood vessels were injected," should be used instead of the word "congestion." This description can be written by anyone, even the laboratory technician, and sometimes it is best to have the description written by some one in this capacity, because there is too much of a tendency on the part of medically trained people to diagnose instead of describe. The number, size, general shape, appearance and differences in appearance of the different parts of the gross material are included in this description. This serves as a means of comparison between the record of the operation and any tissue examination that may be made, and makes certain that both are talking about the same material, for it often happens that those who have charge of the tissues in the operating room get the tissues from different cases mixed. If the laboratory has the gross description of the material that is accredited to a given case, however, all such mistakes can be corrected before diagnosis, so there is no chance of confusion later. Second, the part of the gross specimen which should be examined microscopically is determined by directions from the surgeon or interne or by the pathologist. Third, when the slides are made and ready for examination, the section is inspected in the gross by transmitted and refracted light and is described in the gross, giving size, shape, staining reactions, apparent arrangement of tissue and other points that can be made out by the naked eye. Then follows the description of the slide as seen through

the microscope, followed by the diagnosis. Fourth, a copy of this description is kept by the laboratory and a copy attached to the patient's chart or sent to the clinician if it is an outside case.

There may have arisen in your minds a question as to the value of all this and a conclusion that there is a lot of labor entailed that is not justified by the results. If such is the case, I beg to differ with you. From time to time through this paper I have indicated some of the values of this type of a tissue report. These may be summarized as follows: first, it insures an examination of the right tissues in each case. The appearance of these tissues in the gross is a valuable thing to the clinicians, internes and others. By comparison of this gross description with the microscopic description later on, all concerned become better pathologists and better clinicians. The accurate description of the microscopic examination not only gives in a word or two a diagnosis of the most important pathologic findings, but reveals all of the pathology present, and will ultimately enable the pathologist to form more accurate and differential conclusions with regard to certain pathologic conditions. These conditions might be grouped under a class, as, for example, the habits of growth of tumors, the differences in different inflammations of the same organs and parts. By a study of these reports the pathologist is continually training himself to be more accurate, and hence is able to make more valuable diagnoses. It gives the clinician the fullest possible picture of the processes with which he is dealing. By recording the accurate details from which the diagnosis is made the pathologist is protecting himself in case his diagnosis is questioned. A collection of such pathologic reports enables the clinician at the end of the year to review his cases and by comparing the pathology with the clinical findings is enabled to make more accurate diagnoses in the future. And, last but not least, it is building an invaluable library for research work. Any one interested in any type of pathology would be enabled not only to review the data in a given hospital but also to secure from other hospitals reports and material from similar cases, and so have available a large field of examples from which to draw pathologic conclusions. In this way, and in this way only the pathologist renders the present generation, not only in his own immediate vicinity, but throughout the country, a valuable service. He also renders future generations an invaluable service when he collects material which can be used in clarifying and making definite all pathologic queries and concepts.

## DISCUSSION

*Dr. Robert A. Keilty.*—Frozen sections were mentioned in the paper. For the past five years we have made all our routine surgical pathologic sections, averaging about three a day, by the frozen method. We have almost discarded the paraffin method. We make the section on the first day or, preferably, after twenty-four hours fixation in 4 per cent formalin. I put in a plea for this method; the gross appearance is fresh in mind, the report is quickly gotten out of the way, and the clinical service receives the final diagnosis while the case is uppermost in their minds—not days or even weeks later.

*Dr. Ralph G. Stillman.*—Gross examination of specimens, which is the only record for the laboratory of a specimen as it comes from the operating room, should be made with greatest possible care. The gross appearance of specimens rapidly changes, and the appearance after it is fixed is quite different from that of the fresh material. It is important

that gross descriptions should be passed upon by the older man in the laboratory. The majority of our sections are filed in the form of frozen sections. We do make many paraffin sections, but we have no difficulty in getting good frozen ones. Sixty-five or seventy-five per cent of our specimens are reported on, either on the same day or the next day. We have no trouble with the frozen sections in permanent mounts.

*Dr. A. H. Schade.*—I would also like to make a plea for frozen sections. We use frozen section work routinely. We have sections that run back many years; for instance, we have one section that we have had for thirty years. Recently we have been doing quite a bit of work with tissue the size of a pinhead. We mount them in 10 per cent gelatin, freeze and cut, stain with hematoxylin and eosin, and they give good detail. If the frozen section work is done properly, you will get all the data you wish for microscopic diagnosis.

*Dr. James B. Bullitt.*—I would like to learn how a man can make a frozen section the size of a pinhead with any satisfaction. You can do so in paraffin sections very readily. In the matter of time too, I think a great deal of emphasis has been put upon the time in making a paraffin section. Frozen sections are, of course, very much quicker. After the specimen has been fixed a few hours with formaldehyde it does not take over seven or eight hours to finish the section by the paraffin method. The slower method gives better results.

*Dr. Frank W. Hartman.*—I would like to emphasize the relative merits of fixed frozen sections and paraffin or celloidin sections with regard to the pinhead-sized pieces of tissue that have been mentioned. It is difficult certainly to make both types of sections from such a small bit of tissue, and, if the frozen section does not give the desired results, usually there is not enough left to make paraffin sections. I do not believe that it is necessary to adhere to either method for all types of work. We use paraffin routinely for autopsy work and fixed frozen sections usually in the surgical work. Occasionally, however, we would like very much to get a second section from a particular area in the surgical work, and with a paraffin block we could do this to best advantage. In addition, the paraffin blocks can be filed, serving as a safeguard in case the tissues or slides are lost.

*Dr. Leon S. Lippincott.*—I want to say just one word more in favor of frozen sections. We do not have any trouble. We have made it a routine to save our tissues for at least a month, and we throw away only those of no interest. We have no trouble with pinhead pieces of tissue.

*Dr. L. A. Turley* (closing).—I wish to thank the men for the discussion of this paper. There are a number of interesting things that have been brought up in the discussion of frozen and paraffin methods, which is a subject in itself. The main difficulty with frozen work is that it is particularly difficult to get a section without loss of cells. This is especially true unless the specimen has already been fixed or if there is very much edema, or if the tissue is very cellular. You do not get a complete picture. On the other hand, the paraffin method so contracts the tissues, and sometimes ruins them if the reaction of the fixative is not right, that the picture is distorted. You can be sure that all the cells are contracted, some more than others. I have had no difficulty with pinhead specimens. With reference to the point raised about the gross description: I do not mean by that that just any one could come in and make the description, but it is not necessary to have a medically trained man, in fact it is better not to have one so trained for reasons pointed out in the paper. By saving these specimens you are collecting valuable material for research.

# COCAINE-EPINEPHRIN MIXTURE, ITS TOXICITY AND ANTIDOTE*

By Ellison L. Ross, Ph.D., M.D., Chicago, Ill.

MAYER[1] states, "Ross reported that epinephrin greatly increases the toxicity of cocaine for cats by intravenous injection, basing his conclusion almost entirely on the amounts required to stop the respiration. Ross' conclusions throw little light on the therapeutic use of epinephrin with cocaine and the committee wishes to state that epinephrin in very small amounts does not increase the toxicity of procaine when injected into the subcutaneous tissue or about the nerve." Later in the same article it is stated that, "whether in doses of one milligram or less is capable of increasing the toxic action of cocaine appreciably is not known and this question is in great need of investigation." There are gross errors in these statements made by Mayer. First Ross[2, 3, 4] did not base his conclusions "almost entirely on the amounts required to stop respiration," but also upon changes in the blood pressure, the sensations, and visible reactions of people given the drugs. Second, Ross[2] did not state that epinephrin in very small amounts increased the toxicity of procaine, but gave positive evidence to the contrary. Third, Ross[3] has shown the effect of epinephrin in amounts less than one milligram. Adrenalin (1:1000), as stated in the article, was used in the solutions of cocaine in a concentration from 0.2 to 10 per cent. Calculating from the data given in Table V, it is found that epinephrin was used in doses varying from 0.012 to 0.0250 milligrams per kilogram of body weight. Table A contains the calculated amounts of epinephrin given, in addition to the essential data in the original article.

Table A

| PER CENT ADRENALIN* | WITH COCAINE | VOL. SOL. INJECTED PER KG. | MG. EPINEPHRIN PER KG. | MG. COCAINE PER KG. |
|---|---|---|---|---|
| 0.20 | ¼ | 6.0 c.c. | 0.012 | 15.3 |
| 0.40 | ¼ | 5.6 c.c. | 0.032 | 14.1 |
| 1.00 | ¼ | 4.1 c.c. | 0.041 | 10.3 |
| 5.00 | ¼ | 3.1 c.c. | 0.156 | 7.7 |
| 10.00 | ¼ | 2.5 c.c. | 0.250 | 6.3 |

*Adrenalin is 1:1000 as stated in paper, or 1/10 per cent epinephrin.

In view of the decided toxicity of cocaine with adrenalin, an effective treatment should be available. For simple cocaine poisoning Bastedo[5] recommends ice bag to head and whiskey or large doses of bromides. Underhill[6] states that for depression and collapse from cocaine, stimulants such as caffein, aromatic spirits of ammonia, or hot alcoholic drinks are to be given. Sollman[7] directs the use of aromatic spirits of ammonia and caffein, and says that "amyl nitrite has been recommended." Paulson[8] says of acute cocaine

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poisoning that slight attacks of syncope yield to the assumption of a horizontal position, and if there is great pallor, inhalation of amyl nitrite should be tried. Haines[9] states that inhalations of amyl nitrite and hypodermic injections of nitroglycerin are "signally successful." Cushny[10] states that for acute cocaine poisoning amyl nitrite has been advised when the blood pressure seems much elevated.

Since the observations of the author in previous work have noted the extreme changes in blood pressure associated with the corresponding changes in the minimal lethal dose, it was thought well to investigate the action of atropine with the mixture of the two drugs. With the high blood pressure produced by cocaine and adrenalin there was always a decided stimulation of the vagus. With the high pressure and increased period of cardiac relaxation, conditions would be ideal for the development of an acute and fatal cardiac dilatation. Atropine checks the action of the vagus and so might possibly prevent this. Only three animals were necessary to convince us of the worthlessness of atropine as an antidote for the mixture of cocaine and adrenalin. The results are given in Table I.

TABLE I

EFFECT OF ATROPINE ON M. F. D. OF COCAINE-ADRENALIN

| CAT NO. | % COCAINE | % ADRENALIN | % ATROPINE | M. F. D. GRAMS OF COCAINE HCl PER KG. WT. |
|---|---|---|---|---|
| 1 | ¼ | 5 of 1:1000 | .01–½ c.c. per kg. | 0.0041 |
| 2 | ¼ | " " " | " " " " " | 0.0063 |
| 3 | ¼ | " " " | " " " " " | 0.0060 |
| Average | ¼ | 5 of 1:1000 | .01–½ c.c. per kg. | 0.0055 |

Experiments were next made with the nitrites, as they are the most effective for reducing blood pressure and are the drugs recommended by several authorities[7, 8, 9, 10] as antidotes for cocaine alone. Two series of dogs were observed. The first series was given ½ c.c. of 25 per cent solution of chloretone in olive oil per kilogram, intraperitoneally. The animals were then put upon the operating board and given enough ether to allow the attachment of the proper apparatus for making the injections and measuring the blood pressure. The ether was removed, and, after ten minutes' observation, injections with the Woodyatt machine of ¼ per cent cocaine hydrochloride and 5 per cent adrenalin (1:1000) was begun. When respiration stopped the animals were considered to have received the lethal dose. The second series of animals was treated just the same as the first except that after the normal blood pressure had been taken 1/20 c.c. of spiritus glycerilis nitratis was injected intraperitoneally. Tables II and III give the results obtained.

From Table II we find that the average minimal lethal dose of the cocaine and adrenalin mixture for nine dogs was 0.012 grams of cocaine hydrochloride per kilogram of body weight. This indicates that the mixture is not quite as toxic for dogs as for cats. The M.F.D. determined in a previous paper for cats was 0.0077 grams. The average change in blood pressure was enormous, being from 85 to 235 millimeters of mercury.

From Table III, it is found that the addition of nitroglycerin had in-

TABLE II

M. F. D. AND BLOOD PRESSURE FROM COCAINE AND ADRENALIN WITHOUT NITROGLYCERIN

| DOG NO. | % COCAINE | % ADRENALIN | M. F. D. GM. COC. HCl PER KG. | B. P. BEGINNING | B. P. MAX. |
|---|---|---|---|---|---|
| 1 | ¼ | 5 of 1:1000 | 0.015 | 80 | 268 |
| 2 | ¼ | " " " | 0.011 | 70 | 196 |
| 3 | ¼ | " " " | 0.010 | 110 | 244 |
| 4 | ¼ | " " " | 0.015 | 64 | 244 |
| 5 | ¼ | " " " | 0.012 | 108 | 204 |
| 6 | ¼ | " " " | 0.010 | 90 | 210 |
| 7 | ¼ | " " " | 0.014 | 170 | 298 |
| 8 | ¼ | " " " | 0.011 | 80 | 238 |
| 9 | ¼ | " " " | 0.012 | 90 | 212 |
| | | | 0.012 | 85 | 235 |

TABLE III

M. F. D. AND BLOOD PRESSURE FROM COCAINE AND ADRENALIN WITH NITROGLYCERIN

| DOG NO. | % COCAINE | % ADRENALIN | DOSE NITROGLYC. | M. F. D. COC. PER KG. | B. P. BEGINNING | B. P. MAXIMUM |
|---|---|---|---|---|---|---|
| 1 | ¼ | 5 of 1:1000 | 1/20 c.c. per kg. Sp. Nitrog. | 0.012 | 110 | 216 |
| 2 | ¼ | " " " | " | 0.023 | 120 | 212 |
| 3 | ¼ | " " " | " | 0.021 | 170 | 270 |
| 4 | ¼ | " " " | " | 0.014 | 128 | 244 |
| 5 | ¼ | " " " | " | 0.024 | 146 | 234 |
| 6 | ¼ | " " " | " | 0.021 | 72 | 206 |
| 7 | ¼ | " " " | " | 0.019 | 82 | 210 |
| | | | | 0.019 | 118 | 227 |

creased the minimal lethal dose over 50 per cent. The change in blood pressure was still very great, being from 118 to 227. Apparently nitroglycerin did not prevent the development of the maximal blood pressure of which the system was capable.

From a comparison of the results in these two tables it must be concluded that nitroglycerin does not in these doses prevent the development of great rises in blood pressure and that nitroglycerin does reduce the toxicity of cocaine-adrenalin for dogs.

To establish the fact that nitroglycerin is effective in reducing the toxicity of a mixture of cocaine and adrenalin completely, further work was thought necessary. A series of 10 cats was treated in the same manner as the series of dogs, except that ¼ c.c. of a 0.2 per cent suspension of nitroglycerin per kg. was injected in place of the smaller dose and half as much chloretone was given. The results are embodied in Table IV.

The average M.F.D. of cocaine with adrenalin was found to be 0.0147 grams of cocaine hydrochloride per kg. of body weight. Without nitroglycerin the M.F.D. is 0.0077 grams of cocaine hydrochloride per kg. This means that cocaine and adrenalin so administered are approximately half as toxic with nitroglycerin as without it.

All of the work thus far on this problem had been done on animals anesthetized with chloretone while the cocaine-adrenalin mixture was injected intravenously with the Woodyatt pump. It was proposed to give cocaine and adrenalin intraperitoneally to normal cats, to some with nitroglycerin, and to some without it. A series of 5 cats was given 1 c.c. of 3 per cent cocaine

TABLE IV

EFFECT OF NITROGLYCERIN ON M. F. D. OF COCAINE-ADRENALIN

| CAT NO. | % COCAINE | % ADRENALIN | DOSE NITROGLYCERIN | M. F. D. GRAMS COCAINE HCl PER KG. BODY WT. |
|---|---|---|---|---|
| 1 | ¼ | 5 of 1:1000 | ¼ c.c. of 2% per kg. | 0.021 |
| 2 | " | " " " | " " " " " " | 0.012 |
| 3 | " | " " " | " " " " " " | 0.017 |
| 4 | " | " " " | " " " " " " | 0.013 |
| 5 | " | " " " | " " " " " " | 0.015 |
| 6 | " | " " " | " " " " " " | 0.011 |
| 7 | " | " " " | " " " " " " | 0.013 |
| 8 | " | " " " | " " " " " " | 0.017 |
| 9 | " | " " " | " " " " " " | 0.012 |
| 10 | " | " " " | " " " " " " | 0.016 |
| Average | ¼ | 5 of 1:1000 | ¼ c.c. of 2% per Kg. | 0.0147 |

TABLE V

EFFECTS OF A FATAL DOSE OF COCAINE AND ADRENALIN

| CAT. NO. | PER KG. DOSE DRUG | SALIVATION | TWITCHINGS AND SPASTICITY | CONVULSIONS | DEATH |
|---|---|---|---|---|---|
| 1 | 1 c.c. Coc. HCl 3% and Adren. 30% 1:1000 | 10 min. | 27 min. | 80 min. | 2 hr. 20 min. chloroformed after 4 severe convulsions. |
| 2 | " " " " " | 45 min. | 60 min. | 1 hr. 45 min. | Recovered. Severe vomiting at 2 hr. 15 min. |
| 3 | " " " " " | 80 min. | 2 hr. | None | Chloroformed. Much prostrated. |
| 4 | " " " " " | 20 min. | 20 min. | 123 min. | Found dead 8 hr. after dose. |
| 5 | " " " " " | 30 min. | 50 min. | None in 1st 3 hr. | Found dead 8 hr. after dose. |
| 6 | " " " with Nitroglycerin | 70 min. | None | None | Recovered |
| 7 | " " " " | None | 3 hr. 10 min. | None | Recovered |
| 8 | " " " " | None | None | None | Recovered |
| 9 | " " " " | None | None | None | Recovered |

hydrochloride and 30 per cent adrenalin (1:1000) per kg. of body weight. Three died promptly in convulsions; one developed such a hopeless condition that it was chloroformed, and one recovered. The animals trembled and became severely salivated and spastic. Dilatation of the pupils occurred in each case after the first several minutes. Then incoordination of movements developed. Convulsions were brought on by an attempt to move about the cage. The convulsion was always severely clonic throughout the body.

Another series of 4 cats was given the same dose of cocaine and adrenalin just after an intraperitoneal injection of $\frac{1}{10}$ c.c. of spiritus glycerylis nitratis per kg. of body weight. All of these cats recovered. One cat developed some salivation, and another developed some spasticity and twitching. The results are tabulated in Table V. It is clear from the results tabulated here that nitroglycerin prevents death of cats given the usual lethal dose of cocaine and adrenalin.

## CONCLUSIONS

1. Atropine did not reduce the toxicity of cocaine with epinephrin.

2. Nitroglycerin did not reduce in dogs the rise in blood pressure due to injections of cocaine with epinephrin.

3. Nitroglycerin reduced the toxicity about 30 per cent for dogs in the doses used and under the conditions of the tests.

4. Nitroglycerin administered to cats under anesthesia reduced the toxicity of a cocaine-epinephrin mixture approximately 50 per cent.

5. Fatal doses of cocaine-epinephrin for normal cats can be made nonfatal by the administration of nitroglycerin.

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## THE USE OF SODIUM THIOSULPHATE IN DIAGNOSTIC PROCEDURE*

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THE continued use of sodium thiosulphate is confirming all of the early desires of those who predicted its efficacy in the treatment of the metallic toxemias. About a year ago it was used successfully on several cases of lead poisoning following the absorption of lead tetraethyl. The use of sodium thiosulphate in many refineries and on workmen employed at lead smelteries has again indicated its value. Reports from these industrial heads show that its efficacy is satisfactory. The continued application of the drug in arsenic intoxication shows it to be the most satisfactory means of combating this disturbance, and as a result the application of syphilis therapy has been greatly extended. It has been shown by us that the simultaneous use of the drug with the arsenical does not affect the therapeutic activity of salvarsan. There are several reports from abroad showing that sodium thiosulphate may be used simultaneously with neosalvarsan without producing any change in the therapeutic activity.

In previous articles it has been shown that arsphenamine produces a direct action upon the involuntary nervous system.[1] This has been shown by localization of the arsenic, and by leucocyte counts made upon the blood at various intervals. Alteration in the extent of this intoxication may be brought about by means of sodium chloride, glucose, lactose, and a variety of other means, such as changing the balance of the constituents in the blood stream and the tissues.

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Nyiri[2] has developed the use of sodium thiosulphate as a kidney function test. These investigations have shown that when the drug is introduced into the blood stream, 60 to 70 per cent of it is oxidized to sodium sulphate, while the remaining 30 to 40 per cent remains unchanged in the urine, provided there is a normal kidney function. In abnormal kidney function these values fall between 1 and 23 per cent. The technic employed consists in the use of 10 c.c. of a 10 per cent solution injected intravenously after the bladder has been emptied. The urine is then collected every hour for three hours. The thiosulphate excreted is determined by iodine titration. Nyiri states that as a result of 120 experiments the test is practicable, requires small expenditure of time, makes small demand on the patient, and is quantitative. The injections are painless and safe. Eskelund[3] also employed the test in 31 cases with good results and advocates the continuation of the method.

From the diagnostic point of view little attention has been given to the use of sodium thiosulphate. It has been pointed out by one of us[4] that in an intoxication by a heavy metal, there is a comparatively small amount of that metal present in the blood stream or in the urine. This is particularly the case in jaundice, dermatitis, dermatitis exfoliativa, and scleroderma, showing a pigmentation, and the ordinary leather-like development of the skin. In practically every case of this nature, the blood stream and urine may show a low value, except in a few cases where saturation has developed. Under this condition a higher value may be expected in the blood and in the urine.

These patients may develop the intoxication either through the therapeutic application of arsenicals or through the therapeutic application of remedies highly contaminated with arsenic, or through the ingestion of foods containing glucose, soluble cocoa, apples, potatoes, tomatoes, etc. The contamination in this last group is brought about through chemical processes or as a result of the use of insecticides containing arsenic. The possibilities of infant food supplements are also being considered as a cause of scleroderma observed in several children.

The fact that arsenic is an intoxicant to the autonomic nervous system makes it probable that the cellular activity has been modified to such an extent that there is a deposition of the drug with a resulting modification of the properties of a normal skin. The dermatologist readily diagnoses these cases, but is always at a loss as to the proper procedure from a therapeutic point of view.

For diagnostic purposes specimens of blood and urine should be obtained before any injections are made. In order to secure the best results the daily intravenous administration of 0.5 gram of sodium thiosulphate is advisable. A twenty-four-hour specimen of urine is obtained and this is analyzed for arsenic. An occasional specimen of blood is obtained and checked up for the change in arsenic values. An increase of arsenic in the blood and urine is followed by clinical improvement. In most instances a steady increase of arsenic output is noted. This is followed by gradually diminishing quantities until a uniform output is obtained. Under these conditions the pigmentation decreases, the skin gradually becoming normal in texture and feeling.

Under some circumstances the drug in crystal form may be administered by mouth. In treating children, 0.3 gram is given before meals, three times each day. Adults receive one gram three times each day preceding the meal. Intestinal irritation is the only contraindication to the use of the drug. In many instances it behaves as a cathartic.

Only four patients will be used for the purpose of illustrating the many cases that fall into this class. In the first instance, an adult woman illustrates the diagnostic value of the drug. The second is an adult man with the diagnosis of arsenical pigmentation. The third case is that of a child, about four and one-half years old, representing scleroderma with pigmentation. The fourth is a young girl with arsenical pigmentation. The patients are designated as *A*, *B*, *C* and *D* in the tables showing the total solids and the rate of arsenic excretion.

PATIENT *A*.—Diagnosis of scleroderma was made with the origin unknown. Past history of patient showed that she had been feeling normal in every respect. The first symptoms consisted in the feeling of tightness of the skin over both knees, followed by the same condition in the region of the ankles. The lumbar regions next showed involvement with strips of parchment-like zones extending downward and around the body to the abdomen. The neck, wrists, hands and feet became involved. The feeling of the skin was very similar to parchment and the patient was unable to move the hands and fingers readily. Thyroid treatment was given with no results. General condition of the patient was good. Duration from first onset about one year. Examination of the urine showed arsenic *trace* and the blood 0.03. Treatment with sodium thiosulphate by mouth was instituted, giving 1 gram daily. Urine showed the presence of 1.54 mg. of arsenic per 100 grams of dried specimen on the second day. At the end of thirty days, the excretion was 0.60 mg. of arsenic, and at the end of six months' time the value had reached the normal excretion rate in which 0.11 mg. of arsenic was found. The patient was discharged from the hospital with physical condition good and the skin normal in every respect. Further questioning brought out the fact that the patient worked with a furrier and that the absorption had taken place as a result of handling the furs which had been sprayed with arsenic. At the present time it is reported that the patient is well.

PATIENT *A*

| SPECIMEN | DATE | TOTAL SOLIDS | ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN |
|---|---|---|---|
| Blood before treatment | 11/ 9/23 | 22.50 | 0.03 |
| Urine before treatment | 11/ 9/23 | 2.54 | Trace |
| Urine | 11/10/23 | 2.18 | 0.535 |
| Urine | 11/11/23 | 2.05 | 1.540 |
| Urine | 11/12/23 | 1.77 | 1.150 |
| Urine | 11/13/23 | 2.13 | 0.552 |
| Urine | 11/14/23 | 2.09 | 0.611 |
| Urine | 12/16/23 | 1.71 | 0.696 |
| Urine | 12/17/23 | 1.53 | 3.921 |
| Urine | 12/18/23 | 1.91 | 0.496 |
| Urine | 12/19/23 | 2.33 | 0.141 |
| Blood | 12/19/23 | 24.70 | 0.049 |
| Urine | 1/15/24 | 2.61 | 0.230 |
| Urine | 1/16/24 | 2.61 | 0.240 |
| Urine | 1/17/24 | 2.54 | 0.213 |
| Blood | 1/18/24 | 18.28 | 0.140 |
| Urine | 3/20/24 | 2.74 | 0.110 |

PATIENT *B*.—Roumanian; age, thirty-nine. Twenty-four years in the United States; occupation: telegrapher. Onset: four years age a group of "pimples" appeared in the left scapular region accompanied by intense itching and burning. The rash spread over this region and involved the labium, the trunk, the anterior aspect of both thighs, both forearms, hands and feet. The rash was characterized by grouped vesicles on an erythematous base. Intermingled with the fresh lesions were scars and pigmentation. The clinical diagnosis was dermatitis herpetiformis.

Two years ago patient began taking arsenic in the form of Asiatic pills, averaging 9 pills a day for four months. He then came to the hospital Dec. 20, 1923, remaining until March, 1924. During that time he received Fowler's solution up to 18 drops a day, and his condition improved steadily. He continued taking Fowler's solution for two months after leaving the hospital, then went back to private physician who put him on Asiatic pills and a tonic. He took 200 of the pills in the next two months. During this time his skin became steadily darker until he was pigmented from head to foot with the exception of the flexor surfaces of the great joints, the axillae, the anterior surface of the elbows, groins and popliteal spaces. In addition to the above-mentioned pigmentation, there was a moist eczematoid dermatitis of the face, ears, neck and genital-anal region. The pigmented areas on the trunk and limbs were covered with small follicular miliary papules which at times showed vesicular formation on the summit. At the back of the neck the skin showed a papulomatous condition; the palms showed keratoses. Clinical diagnosis of an arsenical dermatitis in addition to Duhring's disease was made.

He returned to the hospital in September, 1924, and was immediately given sodium thiosulphate intravenously, 1 gram every other day, receiving a total of 45 injections. In the meantime he had taken 1 gram of the thiosulphate crystals by mouth three times a day after meals. The pigmentation faded gradually and the patient's general condition was much improved. Neurologic tests: negative. Lymph nodes generally enlarged.

The present condition of the patient is satisfactory.

PATIENT *B*

| SPECIMEN | DATE | TOTAL SOLIDS | ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN |
|---|---|---|---|
| Urine | 9/25/24 | 2.64 | 0.10 |
| Urine | 9/26/24 | 2.28 | 0.48 |
| Blood | 9/26/24 | 21.34 | Trace |
| Urine | 9/27/24 | 1.52 | 2.19 |
| Urine | 9/29/24 | 3.18 | 2.63 |
| Urine | 10/ 2/24 | 1.60 | 0.36 |
| Urine | 10/ 4/24 | 1.73 | 0.34 |
| Urine | 10/ 6/24 | 2.12 | Trace |
| Urine | 10/ 8/24 | 1.80 | 0.17 |
| Urine | 10/10/24 | 2.33 | 0.61 |
| Urine | 10/16/24 | 3.96 | 0.12 |
| Urine | 10/17/24 | 2.99 | 0.19 |
| Urine | 10/18/24 | 3.97 | 1.27 |
| Urine | 10/21/24 | 2.75 | 0.05 |
| Urine | 10/23/24 | 4.66 | 0.05 |
| Urine | 10/25/24 | 2.37 | 0 |
| Urine | 10/27/24 | 5.26 | 0 |
| Urine | 10/31/24 | 3.09 | Trace |
| Urine | 11/13/24 | 4.44 | 0 |
| Urine | 11/15/24 | 3.44 | 0.16 |
| Urine | 12/ 2/24 | 5.38 | 0.08 |
| Urine | 12/ 4/24 | 4.99 | 0.08 |
| Urine | 12/ 6/24 | 4.52 | 2.29 |
| Urine | 12/ 8/24 | 5.30 | 1.90 |
| Urine | 2/19/25 | 4.27 | 0.34 |

PATIENT *C*.—Jewish, age, four. Patient has suffered for several years with itching and scleroderma. Patches of pigmentation are found on the neck, the trunk, and the buttocks. These areas of pigmentation closely follow nerve distribution. Sodium thiosulphate was administered by mouth in doses of 0.3 grams three times a day before meals. Patient is still under observation. Marked improvement in pigmented areas is noted.

PATIENT *C*

| SPECIMEN | DATE | TOTAL SOLIDS | ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN |
|---|---|---|---|
| Urine | 12/30/25 | 1.61 | Trace |
| Urine | 12/31/25 | 2.97 | 0.157 |
| Urine | 1/ 1/26 | 3.02 | Trace |
| Urine | 1/ 2/26 | 3.02 | 0.138 |
| Urine | 1/ 3/26 | 1.62 | 0.452 |

PATIENT *D*.—Italian, female, age, eleven. The patient has suffered since infancy with a lichenified papular eruption most marked on limbs but involving trunk and face. Clinical diagnosis: Prurigo hebra.

On January 12, 1925, examination showed the pigmentation with the same description as in case *C*. The same follicular lichenified papules were also present.

Injections of sodium thiosulphate: Marked improvement is noted in the lichenified pigmented eruptions. Patient very markedly improved. Still evidence of prurigo. Last visit 3/18/25.

PATIENT *D*

| SPECIMEN | DATE | TOTAL SOLIDS | ARSENIC IN MG. PER 100 GM. OF DRIED SPECIMEN |
|---|---|---|---|
| Blood | 1/ 5/25 | 22.76 | 0.04 |
| Urine | 1/ 5/25 | 3.23 | 0.08 |
| Urine | 1/29/25 | 2.09 | Trace |
| Urine | 3/ 2/25 | 5.04 | 0.087 |

From the data given above it is apparent that the use of sodium thiosulphate has distinct advantages in confirming some doubtful diagnoses and the etiologic factors involved. A general principle that has been observed in such conditions as described above is that the *blood* and *urine* usually show a value for arsenic that is either zero, trace, or a quantity below the normal. During the past year Myers and Cornwall showed that arsenic is a normal constituent of the body and the embryo as well.

These clinical symptoms as illustrated in the cases above are frequently observed but the etiology has been obscure, the treatment unsatisfactory. As a result of studies in blood chemistry which will be published in another article, the use of sodium thiosulphate has been demonstrated to be satisfactory. Solutions of thiosulphate deteriorate and lose much of their therapeutic activity and for this reason it has been found advantageous to use solutions prepared from sterile crystals. These solutions give the best results. The toxicity of sodium thiosulphate is approximately 6,000 mg. per kilogram of body weight. On this basis it is readily recognized that it is difficult to imagine a condition in which an undue toxicity could arise. In spite of this low toxicity it has been found that doses of 0.5 gram either daily or every other day give more satisfactory results. Large doses produce a

too rapid exosmosis which is followed by an exacerbation. Injections are continued until permanent relief is obtained.

It has been pointed out in earlier publications that arsenic of inorganic origin is more difficult to remove than that which is deposited from the antisyphilitic remedies.

## SUMMARY

Sodium thiosulphate in its active form is useful in the diagnosis of scleroderma in which arsenic is a contributing factor. It is employed in such cases as those in which a pigmentation is observed. Continued use of fresh crystals of sodium thiosulphate releases the arsenic that has been deposited either in the skin, or along the nerve trunks until a gradual return of normal conditions is observed. The parchment-like conditions of the skin disappear under treatment and the pigmented areas disappear. A branny desquamation very frequently accompanies clinical improvement. Thus far no evidences of kidney dysfunction have been observed. In fact, quite the reverse is noticed, namely a stimulation of diuresis. The ingestion of large volumes of fluid is recommended. The patients respond promply to treatment and the clinical symptoms improve.

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# THE PREPARATION OF SODIUM MORRHUATE*

By R. A. Cutting, M.D., C.M., M.A., Ph.D., Washington, D. C.

SODIUM morrhuate is regarded by many as practically a specific in the treatment of tuberculosis, particularly in so-called "surgical tuberculosis." The substance is not at present available on the American market and must be prepared as needed. In view of certain difficulties which I have experienced in its preparation and the encouraging results which I have obtained with a good product in the treatment of several cases of tuberculosis coming under my care within the last few months, it has occurred to me that lack of a proper supply of the drug may be responsible in considerable measure for the backwardness of the profession generally in establishing the therapeutic value of this interesting substance. Accordingly, the purpose of this communication is to record some observations on the preparation and physical characteristics of the drug, a knowledge of which is an essential preliminary to successful clinical experimentation with it, though I must reserve for a future discussion such clinical data as I myself have been able to collect.

Sodium morrhuate, when properly prepared, is a somewhat bulky, distinctly yellow powder, with a characteristic piscine odor and a greasy, soapy feel. It dissolves readily in water and produces a solution which is light yellow in color, clear as crystal, and which forms a lather, like other soaps, when agitated. Though little emphasis has been placed on the fact by others who have written on the subject, sodium morrhuate is a relatively unstable substance when preserved under ordinary conditions; in solution it changes little in appearance even when kept in untinted glass and exposed freely to the atmosphere, except that, at the end of a few days or a week, a fine, cloudy, white precipitate settles to the bottom of the container. When clinically tested, the solution does not remain therapeutically active much longer than two weeks even when kept in amber bottles, sterile, and tightly sealed, distinct deterioration of the drug being apparent often within four or five days. The powdered substance not in solution turns somewhat darker on standing and, if not kept in a desiccator, tends to become resinous in consistency. The powder seems to deteriorate more rapidly than the solution; at least at the end of a few days it no longer goes completely into solution as formerly, but forms a murky precipitate, however much care may have been taken in the interim to keep it dry and protected from the light.

Because of the difficulty experienced in ridding the substance of water in the final stage of its preparation so as to obtain a fine dry powder, it first seemed reasonable to assume that the keeping qualities of the drug might be enhanced by placing and storing it in a desiccator, away from contact with all moisture, but apparently the substance deteriorates more rapidly under these

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conditions than when preserved in the ordinary way without taking any special precautions. This is also in accord with the observation, previously noted, that watery solutions of the drug do not deteriorate with undue rapidity, even though they are comparatively dilute (3 per cent).

Accordingly, it seems probable that oxidation is the important factor in deterioration, and it would therefore seem necessary to protect the drug by all feasible means from oxidation both during and after preparation. This end may be accomplished by simplifying and expediting the preparation of the drug, on the one hand, and by final preservation in an oxygen-free medium, on the other.

The technic for the preparation of sodium morrhuate was devised by Sir Leonard Rogers, but he has nowhere recorded, nor has anyone else so far as I am aware, a sufficiently detailed account of the process to enable one not particularly adept in chemical procedures to prepare the substance without considerable experimentation. A more or less complete and detailed account of the process, as I have used it, follows:

Twenty gm. of sodium hydroxide are weighed out with rough accuracy and are dissolved in water to make 150 c.c.; 200 c.c. of 95 per cent ethyl alcohol is added to 100 gm. of cod-liver oil in a 500 c.c. Erlenmeyer flask, and the sodium hydroxide is added, a little at a time, with shaking.

A layer of clear yellow oil now floats on a somewhat darker layer of water, alcohol, and sodium hydroxide. The flask is heated to the boiling point under a reflux condenser, care being taken to avoid bumping, and is kept boiling until saponification of the cod-liver oil is complete, i.e., until the supernatant layer of oil, which grows smaller and smaller the longer the solution boils, entirely disappears, and the contents of the flask assumes a dark amber color; two hours usually suffices for this, though Rogers advocates boiling twice as long.

Twenty per cent sulphuric acid is now added slowly from a burette until a turbid oily precipitate just begins to form and settle to the bottom of the flask; the excess of alkalinity is thus neutralized. Boiling is now continued in the absence of the reflux condenser until most of the alcohol has been evaporated. If carried too far, the solution becomes concentrated, and the flask cracks. At this stage, the solution is thick, dark amber in color, and clear; it is now mixed in a fairly large evaporating dish with 200 gm. of dry, coarse-grained sand, the latter having been previously washed with hydrochloric acid and then with water to neutrality.

The mass is placed on a water-bath and evaporated to dryness as quickly as possible; this takes several hours at best. When dry the plastic sandy mass is placed, a portion at a time, in an extraction thimble and extracted with ether in Soxhlet's apparatus. The ethereal extract is dark amber in color; it should be clear except for a colorless, gelatinous, stringy precipitate which appears when moisture is present in the substance extracted, the precipitate representing water-soluble substances which come through the thimble only to precipitate out again in the ether of the flask. This precipitate really does no harm because it is removed during washing incident to the next procedure. The ethereal extract is next poured into a fairly large separatory funnel, and an excess of 20 per cent sulphuric acid is added to liberate the fatty acids; the amount

needed is variable, but the presence of an excess acidity is readily determined by allowing a drop to fall from the bottom of the separatory funnel onto a strip of litmus paper. The fatty acids are now thoroughly washed with from three to five portions of distilled water until the wash water is no longer acid to litmus paper; this is readily accomplished by allowing the fatty acids to settle out in a distinct layer on top of the wash water and then draining away the latter. The fatty acids during this process appear as a clear oily solution only in case sufficient ether is present to keep them completely dissolved; otherwise they tend to separate out as a solid.

The washed fatty acids are now delivered into a medium-sized evaporating dish, 100 c.c. of alcohol is added, followed by a saturated solution of sodium hydroxide, a drop at a time, to the point of exact neutrality to phenolphthalein. The latter, if added directly to the solution, gives the final product a dark yellow or light brown color; both theoretically and practically this appears to do no harm, though the presence of this substance in the sodium morrhuate is not particularly to be desired; furthermore, the use of an indicator is really unnecessary, since during the process of neutralization the solution itself undergoes a delicate change of color at the end point. Even with the use of phenolphthalein it is not necessary, of course, to add the substance directly to the solution, a drop-reaction plate being at hand.

The evaporating dish is now placed on the water-bath, and the soap evaporated to dryness, the sides of the dish being scraped occasionally with a spatula. This drying takes an hour or more, and, even though the heating process be continued for several hours, it usually becomes necessary to place the product in a desiccator overnight before it can be properly powdered with mortar and pestle. Prolonged heating on the water-bath and prolonged drying in the desiccator must be avoided, both causing very perceptible darkening of the product; a preparation which may be virtually white in a semi-dry condition may rapidly become darker in color as a result of excessive heating, and at the end of twelve hours may have become so dark a brown as to render it presumably unfit for use.

In outline, then, the above process consists in the saponification of cod-liver oil in the presence of alcohol by means of sodium hydroxide and heat, neutralization of the excess of hydroxide, extraction with ether, separation of the fatty acids with an excess of acid, purification by washing with water, and, lastly, resaponification with sodium hydroxide to the point of neutrality.

While theoretically simple and technically by no means difficult, this method yields results which are not uniform. The fatty acids entering into the final product are mainly unsaturated, and very little is known concerning their physical or chemical properties; presumably they occur in different relative and absolute proportions and amounts in different samples of oil, though this presumption hardly seems to account for all the difficulties sometimes experienced in preparing a first-class product.

Reference has already been made to the instability of sodium morrhuate under ordinary conditions, but I have discovered that if it be sealed in a glass tube under a vacuum it keeps much better, since by this method oxygen is excluded. This may readily be accomplished by weighing out convenient

amounts of the substance into test tubes of small diameter, heating the glass tube a suitable distance above the upper level of the powder, drawing out the tube quite thin, attaching a Chapman pump, creating as complete a vacuum as possible, and then applying the point of a flame to the most constricted part of the tube to complete a seal.

Since it seems almost certain that prolonged heating during any part of the above-described process interferes with the efficacy of the drug, and since that portion of the technic which calls for drying after mixing with sand entails by far the longest heating, any modification of the process which would do away with this step would appear to be highly desirable. A convenient means for accomplishing this is to "salt out" the soap by saturating the solution first obtained as a result of saponification (after neutralizing the excess of hydroxide) with sodium chloride; the soap is thus formed immediately as a sticky, semisolid mass from which the excess of water is readily expressed. complete drying on the water-bath being readily accomplished. The crystalline sodium chloride serves as an excellent substitute for sand, so that the ether extraction is accomplished without delay. This modification I regard as highly valuable, and I now use it in preference to that previously described. The sodium chloride is insoluble in ether and remains behind in the extraction thimble of the Soxhlet, and such small amounts as might find their way through the thimble are, of course, subsequently removed during the process of washing the fatty acids.

For clinical use sodium morrhuate is dissolved in distilled water in 3 per cent solution and placed in ampules or vaccine bottles and sterilized; tricresol may or may not be added. Injections are best made intravenously, starting with 0.1 to 0.3 c.c. and increasing by 0.1 c.c. with each dose to a maximum of about 1.0 c.c.; injections are given at intervals of from three to five days. Fresh solutions should be prepared at least as often as once a week.

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# INABILITY TO ESTABLISH THYROID TOLERANCE IN WHITE RATS*

By J. Alexander Clarke, Jr., M.D., Philadelphia, Pa.

CARLSON, Rooks and McKie[1] in early experiments on thyroid intoxication in various animals mention the possibility of establishing a tolerance to thyroid extract. The present experiment was started to obtain some information about such a tolerance if existant. None could be demonstrated as a result of thyroid finding, but, rather, a condition of hypersensitiveness appeared to be developed.

Thirty-two young rats of about 100 grams, the offspring of tumor-bearing rats, were selected. Of these twenty were males and twelve females. They were kept in two cages, males and females being separated. The diet was to consist, in addition to the thyroid, in unlimited bread and milk. This diet was the same as that used for the tumor-bearing rats in adjoining cages, which rats were well and healthy except those in the last stages of large tumor growth. It was soon found that this diet was insufficient for the thyroid fed rats, due, presumably to the greatly increased appetites of these rats. Before this condition was realized and remedied, five had died, two controls and three experimental rats. It may also have hastened the death of some of the experimental rats later in the experiment.

The thyroid used was commercial, dried extract in powdered form from two reliable companies.† The doses were weighed individually on an analytical balance and mixed with bread crumbs and water to form a paste. Feeding was generally easy, especially after the thyroid had been administered a few days. The appetite soon became voracious and continued so until death. One death occurred within a half hour after eating. There were two exceptions to this and in both cases the experiments had to be stopped because the rats refused to eat more thyroid.

The symptoms noted were the same as those of other observers. The earliest was an increase in the appetite. The fur became rough and the rat became thin without much loss of weight. This thinness was particularly apparent along the spinal column, which became very prominent. Shortly before death the rats became restless, often developed a diarrhea, lost weight rapidly, became too weak to stand, and died. If one waits for a pronounced loss in weight as an index to toxemia, the rat will usually die. Recovery after cessation of thyroid feeding is remarkably quick, the rat usually gaining 10 to 20 gm. in a week; the diarrhea subsides rapidly and the rat becomes normal in appearance. The increase in the susceptibility to infection was also noted. There were infections in the thyroid rats and none in the controls.

*From the Laboratory of the Philadelphia General Hospital.

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†The iodine content of these two powders was, unfortunately, not obtained. The change was made on March 21, and, since there is no decided change in the curves at this date, we assume they were of approximately the same strength. There were four deaths from the first and six from the second powder.

Three methods were tried to raise the tolerance. First, to see if one attack of toxemia provided any protection against a second poisoning. Second, to start with a dose that was tolerated and to increase this dose gradually. Third, to give doses that were tolerated at intervals of one week for a long period before starting the toxic doses.

EXPERIMENT 1.—Starting Feb. 9 five rats were fed a concededly toxic dose of 0.5 gm. daily. On Feb. 24 one rat had diarrhea and had lost 25 gm. Feeding of this rat was then stopped. On March 1 another rat died after losing 35 gm. in weight. Of the other three rats, on March 1, one was at the original weight while the other two had lost 15 and 20 gm. each. Feeding of all rats was then stopped until March 11. At this time all four rats appeared healthy and had gained considerable weight (smallest 25 and largest 35 gm.). Thyroid feeding was then resumed, 0.5 gm. daily. On March 22 the rat which showed the first symptoms of intoxication died after a loss of 30 gm. His first feeding lasted fifteen days and he still lived, the second feeding lasted about eleven days and ended in death. On March 29 two rats died after a loss of 10 and 35 gm., respectively. This

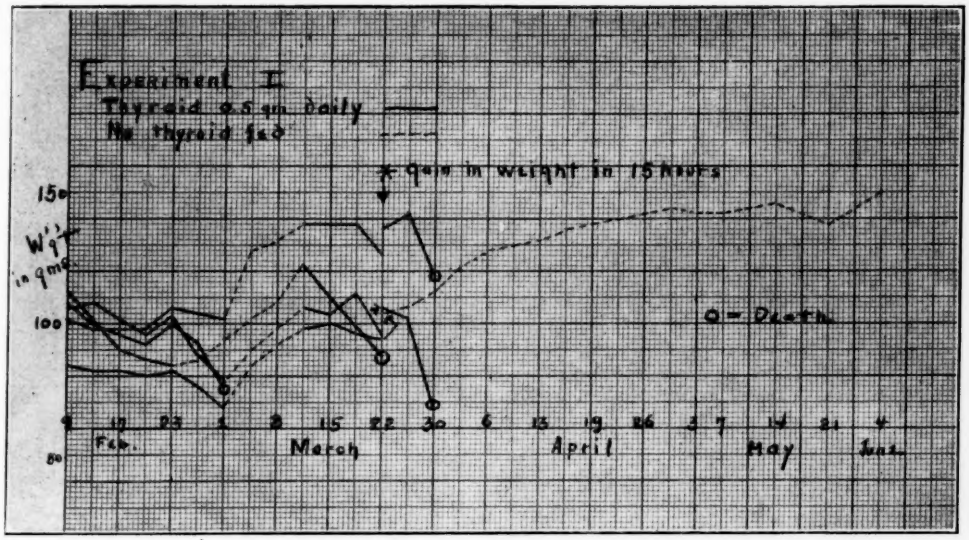


Chart I.

feeding lasted eighteen days, while the first lasted twenty-one days. The feeding of the remaining rat was stopped, but the feeding had been so difficult during the last week it is doubtful whether this last week should be considered a part of the experiment. There was a gain of about 10 gm. in weight during the period. When last seen on June 4 the rat weighed 150 gm. and seemed none the worse for two thyroid intoxications.

EXPERIMENT 2 was similar to experiment 1 except that 0.1 gm. of thyroid was fed daily instead of 0.5 gm. Feeding was started on Feb. 9 in all rats. On Feb. 21 and Feb. 23, respectively, two rats died and another rat showed such a great loss of weight that thyroid feeding was stopped on Feb. 18. Since it was during this period that one of the control rats died, it is probable that these deaths were either due wholly to the feeding deficiencies mentioned above, or were largely influenced by them. In any event they should not be considered thyroid deaths. The rat whose feeding was stopped on Feb. 18 gained 35 gm. in weight and was again fed daily doses of 0.1 gm. from March 1 until he died, April 5. Three days before death there had been only a loss of about 7 gm., although other evidences of thyroid intoxication were present, notably weakness. This 145 gm. rat took thyroid extract, 0.1 gm. for 35 consecutive days, before succumbing. This record is only surpassed by one other rat, and this was a 200 gm. rat.

Of the remaining rats in this experiment, one died on March 1 after being fed 0.1 gm. since Feb. 9, with a loss of 25 gm. Feeding was stopped on the other rat on March 1, during which period there had been a loss of 20 gm. At this time the rat was not considered in a dangerous condition. On March 11, after a gain in weight of about 18 gm., feeding was again started at 0.1 gm. daily. On March 30 the rat was very weak, had diarrhea, and, although there was a gain of almost 10 gm. in weight for the period of feeding, the feeding was stopped. Recovery was rapid (a gain of 20 gm. in the first week), and, when last seen on June 4, was alive and healthy, having gained 75 gm. This rat then had two thyroid feedings with an interval of nine days between. The first lasted twenty-one days without alarming symptoms, while the second lasted twenty days and left the rat in a dangerous condition.

EXPERIMENT 3 was designed to start at a dose below a toxic dose and endeavor to show tolerance by gradually increasing the dosage. Accordingly, the five rats were fed 0.01 gm. thyroid daily, this being approximately 1/10,000 of their body weight.

Of the five rats used in this experiment, one was so very obviously abnormal that it should not be considered in the experiment. This rat weighed 10 gm. less on June 4 than

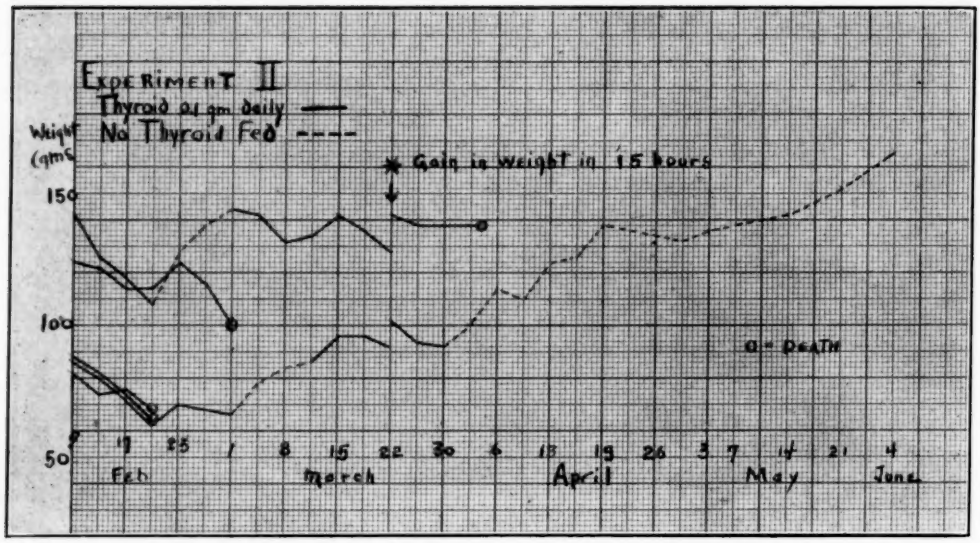


Chart II.

it did at the start of the experiment on Feb. 9. In this rat 0.01 gm. of the thyroid caused a loss of weight of 30 gm. in twenty-eight days. For seven days 0.02 gm. was fed, when the rat became very weak and had diarrhea. After this there was only a 20 gm. gain in weight, ten of which was lost. For some unknown reason this rat either was more than ordinarily susceptible to thyroid or the synchronism of the alarming symptoms with the thyroid feeding was a coincidence.

The other rats were all fed on the same schedule, to wit: 0.01 gm. daily for fifty-three consecutive days, Feb. 9 until March 23; 0.02 gm. for seventeen days, March 23 to April 6; 0.04 gm. for seven days, April 6 to April 13; 0.07 gm. for seventeen days, April 13 to April 30.

During the first period all rats thrived, showed no symptoms of thyroid poisoning, and there was a gain in weight, 30, 28, 8 and 5 gm., respectively. During the same period, the five surviving control rats gained 65, 38, 20, 20, 8 gm., respectively, which about proves that 0.01 gm. is a harmless dose for rats.

During the second period the rats gained as follows (same order as above) 20, 0, 8, 0 gm. During the third period, 0.04 gm. daily, there was no gain in weight nor was there any loss.

During the last period, 0.07 gm. daily, one rat died April 25, showing no lose in weight nor any of the symptoms of thyroid intoxication. The last weight recorded was the death weight. The three remaining rats all showed a loss of weight, 15, 15, and 20 gm., respectively, although none had diarrhea. Feeding was then stopped and was immediately followed by a very rapid gain in weight, at least 20 gm. in the first week, which is very fair proof that all were suffering from too much thyroid. When last seen on June 4 they had gained 60, 40 and 50 gm., respectively.

This experiment offers more hope of establishing tolerance than either of the two preceding ones. It is possible that the definite loss of weight without the diarrhea noticed in the other rats may have been an evidence of tolerance to these symptoms. Had the increase been more gradual the results might have been different.

EXPERIMENT 4 was originally planned to produce a case of thyroid intoxication by large, single, weekly doses and then to see if this intoxication would prevent further intoxication with the same doses. Accordingly 5 rats were fed 1.0 gm. in one dose once a week. In some instances it was necessary to take two days to the feeding, but in every

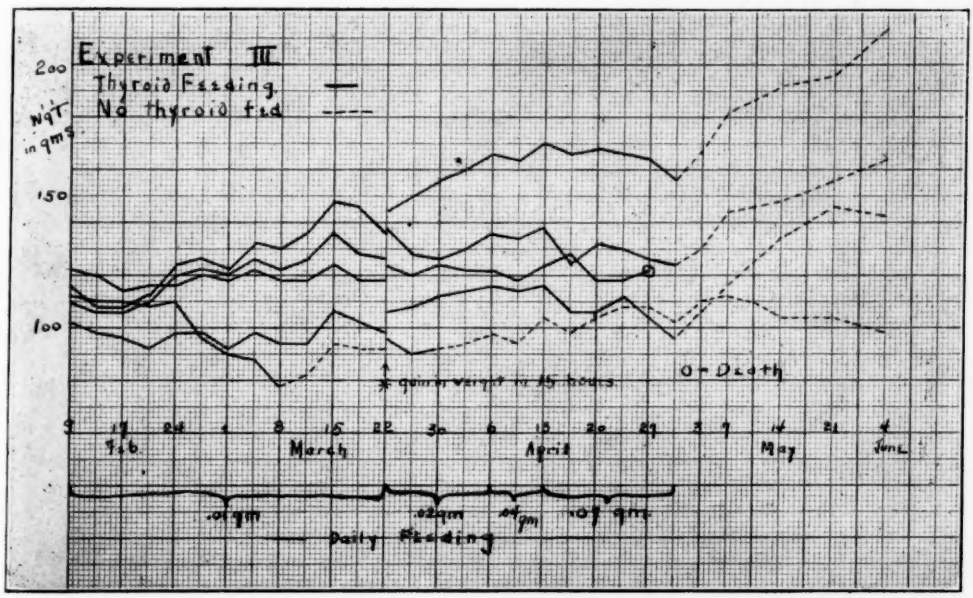


Chart III.

case the full gram was given in not less than two consecutive days. We were greatly surprised to find that all five rats thrived and gained weight on 1.0 gm. in a single dose when 0.7 gm. weekly in seven doses consistently produced alarming symptoms or death. The dose was then divided and fed at intervals of less than a week, keeping the total weekly dose approximately the same. When 0.3 gm. every other day was reached, the symptoms of intoxication appeared in all rats.

There were three periods of feeding, the first 1.0 gm. weekly from Feb. 9 to March 30, 50 days; the second from March 30 to April 20, 21 days; and the third from April 20 to April 30, 10 days.

During the early part of the first period the gain in weight was not great, and in two rats there was some loss. The rather rapid gain during the last part of this period may be an evidence of tolerance, but, since the feeding conditions were also improved at this time, the evidence is far from conclusive. The gains during the whole period were as follows: 50, 43, 23, 15, and 15 gm. During the second period 0.5 gm. was fed twice a week, and the weights remained about stationary. During the third period all rats lost weight except one, the losses being 15, 13, 10, 7 gm. One rat absolutely refused to eat any more

thyroid and feeding was discontinued on April 27. Within a week this rat had gained over 30 gm. Of the remaining four rats three made the usual rapid recovery immediately upon the withdrawal of the thyroid, although the actual weights were not so great, chiefly because of the fact that these rats had already attained their full growth when the intoxication began. One rat, however, did not gain upon the withdrawal of the thyroid and died April 7 with bloody diarrhea, one week after stopping the thyroid. This was the only case in which recovery did not occur if the rat lived twenty-four hours after the last dose.

EXPERIMENT 5.—In this experiment 0.1 gm. was fed once a week from Feb. 9 to March 23, 45 days, and then 0.1 gm. daily until symptoms of intoxication appeared. One rat died Feb. 23, probably because of the early feeding difficulties mentioned above. During the first, the period of weekly feeding, the rats gained as follows: 60, 50, 20 and 10 gm., respectively. After 14 days of 0.1 gm. of thyroid daily the two rats which had made the small gains were very weak and the feeding was discontinued. Both of these rats made

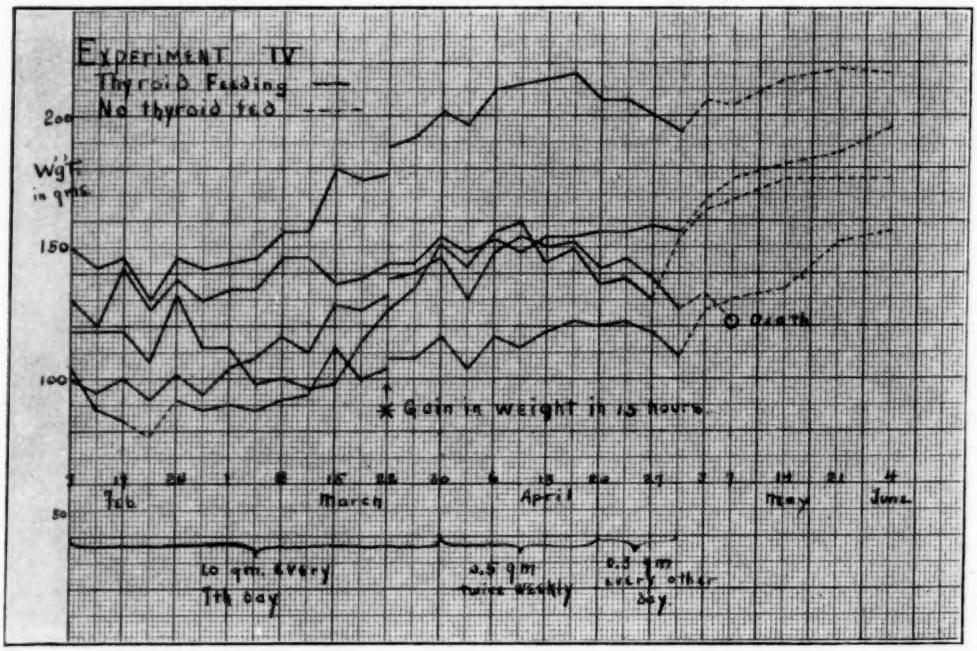


Chart IV.

the usual quick recovery and subsequently became normal healthy rats. There was very little loss in weight in the two remaining rats, about 8 and 12 gm. One rat took 0.1 gm. daily until April 16, 24 days, at which time it had lost 30 gm. in weight and was very weak. The remaining rat took 0.1 gm. daily until April 30, when the feeding was stopped, a period of thirty-eight consecutive days. At this time the rat had lost but 20 gm. in weight and was not considered in a serious condition. There was the usual rapid gain, however, 20 gm., during the first week after withdrawal. This was the longest consecutive period of feeding and may be accounted for by the fact that this rat had gained so rapidly on 0.1 gm. weekly that it weighed 200 gm. at the onset of the daily feeding period. Certain it is that none of the other rats in this group showed any increased tolerance as a result of their previous weekly dosage, and this rat was not insusceptible, as is shown by his rapid increase in weight after the thyroid was stopped.

Recovery after thyroid feeding is stopped is so rapid that it is worthy of special mention. This was especially noticeable in this experiment, because the rats were about half grown. While being fed thyroid the rats continued their growth but were quite devoid

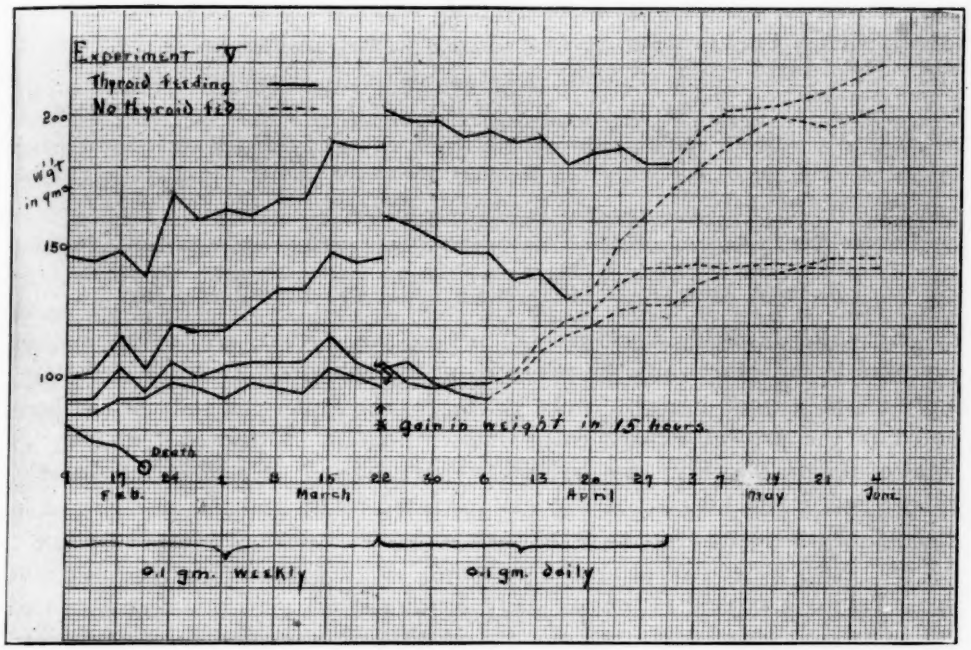


Chart V.

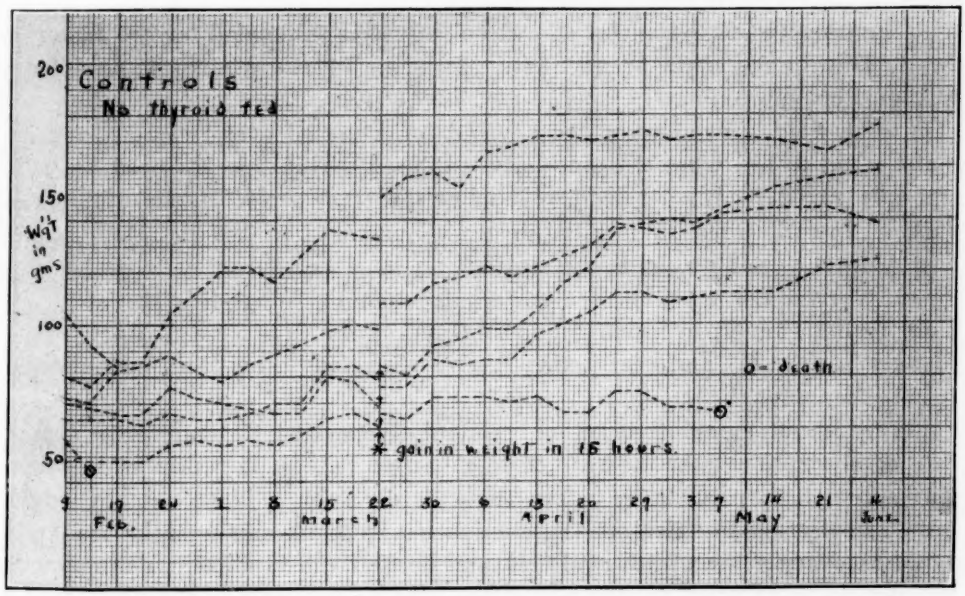


Chart VI.

of fat. This fact, in some of the rats, made the loss of weight appear trifling. For the same reason the gain in weight afterwards was even more dramatic.

There were 27 instances of poisoning in 21 rats with 19 recoveries and 8 deaths. Of those that died, all but one died within thirty hours of the last feeding. One rat lived for a week after the last dose, during which time it had a bloody diarrhea dating from before the last dose of thyroid. (Three rats died in the second week of feeding, and these deaths were not considered as due to thyroid.) (See Table I.)

TABLE I

GAIN IN WEIGHT AFTER CESSATION OF THYROID FEEDING

| GAIN IN GRAMS | GAIN IN FIRST 3 DAYS | GAIN IN SECOND 3 DAYS | GAIN IN SECOND WEEK | TOTAL GAIN IN 2 WEEKS |
|---|---|---|---|---|
| 10 or less | 6 rats | 10 rats | 8 rats | |
| 10–20 | 10 | 9 | 10 | |
| 20–30 | 3 | | 1 | 9 rats |
| 30–40 | | | | 8 |
| 40–50 | | | | 2 |

ON THE MECHANISM OF THYROID POISONING IN WHITE RATS

Kendall,[2] in 1917, working with goats found that, while 150 mg. of thyroxin by injection could be tolerated at a single dose, 11 doses of 0.1 mg. daily killed, a total dosage of only 110 mg.[3] The same phenomenon has been noted in the present experiment. One-tenth gram of thyroid fed daily (a total of 0.7 gm. weekly) is a positively toxic dose of thyroid in all the nine rats so fed, while 1.0 gm. weekly was tolerated easily and the rats gained in weight. Five-tenths gram twice a week in five rats did not seem toxic, while 0.3 gm. every other day produced poisoning in all five rats and the death of one. Taking a typical rat on the daily feeding of 0.1 gm., it is seen that this rat in twenty-seven days took 2.7 gm. of thyroid and died, while five rats, for the same period of twenty-seven days, took 4.0 gm. of thyroid in four doses at weekly intervals, thriving and gaining in weight during this period.

The toxicity of thyroid for the white rat seems to depend on two factors:

1. The dosage must be adequate. In some of the present experiments 0.01 gm. or approximately $\frac{1}{10,000}$ of body weight, four rats were able to gain some weight, even when fed thyroid for a period over twice as long as that needed to kill when 0.1 gm. daily was used. This was followed by 0.02 and 0.04 gm. daily for a period as long as the fatal period in the other rats without any fatalities. When the dose was increased to 0.07 gm. daily there was distinct poisoning, with one death.

2. The dosage must be of a certain frequency. What this frequency is must be determined by further experiments. Four rats were distinctly poisoned when fed 0.3 gm. every other day, whereas we know that once a week is not often enough.

This is not cumulative action. Cumulative action depends on a faster rate of absorption than the rate of elimination. In all instances of cumulative action it is possible to poison immediately with a large enough single dose. In thyroid intoxication the elimination is rapid, as shown by the very rapid recovery after withdrawal. It may be that the rapid elimination saves the rat, when fed the single large doses, and that the thyroid is eliminated before the tissues have a chance to absorb and be poisoned by it. This does not explain why the tissues are slow in taking up the thyroid when presented in the larger doses.

A somewhat similar action is seen when iodides are administered over a long period in human beings. Iodide can be taken over a period of two or three weeks, and then suddenly acne will develop and this, in spite of the fact that iodide is one of the easiest drugs to eliminate.

The question as to whether this is not a hypersensitive phenomenon might very properly be asked. Anaphylaxis in the dog and rabbit is produced after the repeated injection of small nontoxic doses. In the rat there is, of course, no rapid and dramatic shock as is seen in anaphylaxis, and it is not intended to suggest that we have anaphylaxis here. If this is a form of hypersensitiveness, it is a form which does not appear in any of the classifications of this interesting condition. It also resembles roughly the human form (called by Coca, atopy) as manifested by asthma and hay fever. It is not at all uncommon to find adults of twenty-five and thirty years of age becoming suddenly sensitive to the feathers on which they have slept since childhood. The reason for this development of hypersensitiveness after such prolonged exposure is not at all understood, the only information we have being the hereditary factor demonstrated by Cooke and Vander Veer.[4]

## OBSERVATIONS

1. No tolerance to thyroid extract in the white rat has been shown in the present experiments, nor do these experiments entertain much hope that such a tolerance is possible.

2. A dose of thyroid, easily tolerated when given in weekly doses, will kill when given in seven doses at daily intervals. Possible explanations of this are: (1) that the thyroid is not absorbed but passes through the bowel unaltered. This does not seem compatible with the digestive powers of the rat. One day the rats were weighed while quite hungry and were then given all the food they would eat and weighed again the next day. The results are shown in the charts as two weights on the same day (indicated by asterisk). It will be seen that there is a gain in weight of from 5 to 15 gm. Since thyroid was usually fed on an empty stomach it does not seem reasonable to suppose that 1 gm. of so easily digestible a substance as thyroid would fail to be digested and absorbed. Furthermore, Kendall, working with thyroidin, and Hunt, with thyroglobulin, noticed similar results, and their dosage was administered subcutaneously or intravenously. (2) That the tissues are unable to take up enough thyroid after a single dose before its excretion to produce a toxic effect. (3) That daily dosage of thyroid produces in the tissue a hypersensitiveness to thyroid of a type not at present found in any classification of hypersensitiveness.

## REFERENCES

[1]Am. Jour. Phys., 1912, xxx, 129.
[2]Kendall: Mayo Clinic, 1917, ix, 330.
[3]Compare Hunt, Arch. Int. Med., 1925, [illegible], 682, who notices a different action of thyroid in single large doses and small divided doses in mice.
[4]Jour. Immunol., 1916, i, 201.

# THE RENAL FUNCTION INDEX*

## A Simple Method for Determining the Functional Activity of the Kidneys

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VARIOUS methods have been advanced for determining the functional activity of the kidney. Most of them have involved the use of complex formulae, and while at first accepted with great enthusiasm, they have either been discarded or replaced by other formulae of equal complexity.

In 1909 Ambard[1] and his collaborators advanced their ideas regarding the rate of urea excretion, with reference to the concentration of urea in the blood and urine, and proposed the rather complex formula frequently referred to as Ambard's coefficient. In 1915 McLean[2] modified this formula by introducing certain additional factors. In 1921 Austin, Stillman, and Van Slyke[3] published a formula which differed considerably from that of Ambard and McLean, while later, Addis and Drury[4] suggested an index which expressed as a ratio the urea in one hour's urine and the urea in 100 c.c. of blood. This later method, while simple, is not sufficiently accurate because of variations which occur in the hourly urinary excretion.[5]

In 1924 Stander, Duncan, and Moses[6] reported on the excretion rate of urea in the toxemias of pregnancy, using the indices of Addis and Drury[4] and of Stillman, Austin, and Van Slyke.[3] These results were compared with a third formula proposed by them, viz.: $X = \frac{B. \times T.N.}{D}$ *B* represents the urea nitrogen of the blood expressed in milligrams per 100 c.c., *T.N.* the total nitrogen in the urine expressed in grams per twenty-four hours, and *D* the urea excretion expressed in grams per twenty-four hours. The formula of Stander, Duncan, and Moses[6] consists of a ratio, expressing the relation between the blood urea nitrogen and the urine urea nitrogen (expressed as a percentage of the total nitrogen of the urine).

Stander, Duncan, and Moses[6] were unable to obtain uniform results for the urea excretion rate by using the formula of Addis and Drury, but did obtain fairly constant results with that of Stillman, Austin, and Van Slyke. Their new index, however, afforded in general even more constant values than the formula of Stillman, Austin, and Van Slyke.

We have used the index of Stander, Duncan, and Moses in a study of sixty cases of normal and abnormal kidney function in an attempt to establish the normal index and its variations in pathologic conditions.

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### PROCEDURE

The urine is collected for twenty-four hours, at the conclusion of which the blood specimen for urea nitrogen (Van Slyke-Cullen) is obtained while the patient is in the fasting condition. The total nitrogen (Kjeldahl), urea nitrogen, and ammonia nitrogen of the urine is determined. During the time of the test the patient is not subjected to any restrictions.

### CALCULATION OF THE NORMAL INDEX

A normal adult, of medium size, on a mixed diet, will excrete 12 to 18 grams of nitrogen during twenty-four hours. The urea excretion depends entirely upon the absolute amount of total nitrogen excreted and the mean average daily excretion in normal adults is 80 to 90 per cent of total nitrogen. The blood urea nitrogen in normal individuals varies between 12 to 15 mg. per 100 c.c. of blood. From these assumptions we can calculate the normal index as follows:

If we take the high normal total nitrogen elimination as 18 gm. in twenty-four hours, assume that 80 per cent is excreted as urea (14.4 gm.) and use the high normal blood urea nitrogen of 15 mg. per 100 c.c. in the formula, the index would be 18. Using 12 gm. total nitrogen excretion in twenty-four hours as the low normal, 80 per cent of which (9.6 gm.) is urea and the low normal blood figure of 12 mg. of urea nitrogen, the index would be 15.

It can can be readily seen from the factors entering into the calculation of the index, that several combinations exist in which changes of the index may occur. Only four of these are of clinical interest.

*A* { Increase of blood urea nitrogen
Normal total nitrogen excretion
Normal urea nitrogen excretion

*B* { Normal blood urea nitrogen
Normal total nitrogen excretion
Low urea nitrogen excretion

*C* { Normal blood urea nitrogen
Low total nitrogen excretion
Low urea nitrogen excretion

*D* { Increased blood urea nitrogen
Decreased total nitrogen
Decreased urea excretion

We are chiefly concerned with types *A*, *C*, and *D*, while *B* is the type not encountered in nephritis but in cases of poor liver function or improper urea formation.

### CLASSIFICATION OF CASES

Arbitrarily the cases have been classed in three groups according to the index obtained. The first group comprises those with an index up to 18 (the normal group), the second from 18 to 40, and the third over 40. The first group was further subdivided into those cases having a normal index, negative urine and no hypertension (Table I-*A*) and those cases with a normal index, albumin and casts in the urine and hypertension (Table I-*B*).

Group two (Table II) cases show an index between 18 and 40. It is in this group (thirty cases) that the so-called mild nephritics, with normal blood chemical findings, are to be encountered. In several of the patients of this group, follow-up determinations of the renal function index were made at varying intervals after treatment, in order to determine whether clinical improvement was accompanied by a similar improvement in the index. It would be without purpose to discuss all of the thirty cases. Several selected ones will be sufficient to give the general idea.

CASE 6726.—A painter, age thirty-six, complained of pain and swelling of the left knee-joint, which had been preceded by a sore throat, cough and fever. His past history revealed that for several years he had suffered from shortness of breath, particularly after exertion. His physical examination showed carious teeth, enlarged heart with double murmur at the apex, and tenderness of both knee-joints. There was also a generalized glandular enlargement. The diagnosis of acute rheumatic fever with chronic endocarditis was made.

On November 11, 1924, the chemical blood examination showed: urea nitrogen 15.6, creatinine 2.3, uric acid 3.3, sugar 138. The urine at this time was negative. On January 6, 1925, another blood examination showed that the blood urea nitrogen had increased from 15.6 to 20.8 and the renal function index determined on that day was 33.4. The urine showed the presence of albumin and hyaline casts. The patient was then placed on a restricted protein diet with rest in bed for about ten days. His rheumatic fever had subsided and he was discharged in good condition. On March 16, the patient returned for another determination of the index. He reported that he had been able to carry on his usual work since discharge and had enjoyed good health. No special restrictions in the diet had been enforced. The blood urea nitrogen at this time was 8.9 and his index had decreased to 11.3. The urine examination was negative.

CASE 7244.—A clerk, age forty-six, gave the following history: A few days prior to admission, he caught cold and developed an acute otitis media. Following this he began to complain of chills and had rusty sputum. His past history was that of scarlet fever during childhood, chancre thirty years ago, and an attack of influenza about four years before admission. He had also been subject to frequent attacks of sore throat. The patient presented a typical pneumonic consolidation of the left upper lobe.

On January 5, 1925, two days after admission, a chemical blood examination revealed a urea nitrogen of 35.7, creatinine 1.3, uric acid 3.5 and sugar 162. On January 8, the

TABLE 1-*A*

NORMAL INDEX, NEGATIVE URINARY FINDINGS

| HIST. NO. | DATE | URINE | | BLOOD | | URINE | | DIAGNOSIS | DIET | INDEX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | U. N. | T. N. | UREA N. | PRESSURE | ALB. | CASTS | | | |
| 6350 | 11/25 | 6.74 | 8.2 | 14.2 | 124/76 | 0 | 0 | Syphilis | Soft | 17.2 |
| 6894 | 12/4 | 7.6 | 10.5 | 11.5 | 104/64 | 0 | 0 | Duod. Ulc. | Ulcer Diet | 15.8 |
| 3663 | 12/6 | 4.81 | 4.97 | 16.6 | 112/70 | 0 | 0 | Lung Abscess | Soft | 17.1 |
| 6954 | 12/9 | 5.9 | 6.2 | 7.2 | 120/90 | 0 | 0 | Neurosis | Norm. | 7.4 |
| 6698 | 12/15 | 2.95 | 4.47 | 8.9 | 110/80 | 0 | 0 | Arthritis | Soft | 13.4 |
| 6762 | 12/20 | 4.9 | 7.03 | 10.0 | 90/60 | 0 | 0 | Duodenal Ulcer | Ulcer Diet | 14.3 |
| | 1/13 | 5.7 | 7.0 | 11.4 | " | 0 | 0 | " " | " | 14.0 |
| 7223 | 1/5 | 7.62 | 8.2 | 10.5 | 100/64 | 0 | 0 | Hodgkin's Convalesc. | Full Semi-solid | 11.2 |
| 7155 | 1/12 | 6.5 | 9.0 | 12.5 | 104/80 | 0 | 0 | Pneumonia Chr. Valv. | | 17.2 |
| 1571 | 1/15 | 6.4 | 10.0 | 11.4 | 138/80 | 0 | 0 | Disease | Soft | 17.8 |
| Mrs. J. | 1/31 | 9.4 | 11.8 | 9.2 | 120/80 | 0 | 0 | Pregnancy Ac. Rheum. | Norm. | 11.5 |
| 8115 | 4/6 | 5.4 | 7.2 | 12.5 | 150/90 | 0 | 0 | Fever | Soft | 16.6 |
| 6730 | 4/25 | 5.0 | 5.9 | 14.8 | 102/65 | 0 | 0 | Nephritis | Milk | 17.4 |

TABLE 1-*B*

NORMAL INDEX, POSITIVE URINARY FINDINGS

| HIST. NO. | DATE | URINE | | BLOOD | | URINE | | DIAGNOSIS | DIET | INDEX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | U. N. | T. N. | UREA N. | PRESSURE | ALB. | CASTS | | | |
| 7105 | 1/15 | 2.7 | 4.0 | 10.9 | 170/64 | +++ | Hyal. Gran. | Chronic Nephritis | Low Prot. | 16.1 |
| 7537 | 2/6 | 8.1 | 8.5 | 9.6 | 110/80 | + | Hyal. Gran. | Chr. Valv. Disease | Karell | 10.2 |
| 7580 | 2/11 | 11.3 | 13.1 | 12.5 | 180/100 | + | Hyal. | Chr. Nephritis | " | 14.5 |
| 7633 | 2/13 | 5.5 | 7.4 | 8.0 | 225/120 | ++ | Hyal. | Cardiorenal Dis. | Soft | 10.8 |
| 7599 | 2/28 | 10.3 | 12.5 | 13.0 | 160/90 | + | Gran. Hyal. | Chronic Nephritis | " | 15.7 |
| 7751 | 3/5 | 4.2 | 5.9 | 12.5 | 150/100 | +++ | Gran. Hyal. | Cardiorenal Dis. | " | 17.5 |
| 7808 | 3/6 | 6.5 | 8.4 | 13.0 | 115/75 | + | 0 | Hyperthyroid | " | 17.5 |
| 5925 | 3/27 | 4.8 | 5.5 | 9.2 | 180/110 | + | Hyal. | Cardiac Insuffic. | Milk | 10.5 |
| 8018 | 3/31 | 9.5 | 12.9 | 14.7 | 105/65 | + | 0 | Tbc. Pneumonia | Soft | 17.8 |
| 7986 | 3/31 | 7.4 | 8.6 | 12.5 | 125/55 | + | Hyal. | Hyperthyroidism | " | 14.5 |
| 7944 | 4/9 | 3.4 | 4.2 | 13.8 | 110/60 | + | Hyal. | Acute Rheumatic Fev. | Fluid | 17.0 |
| Dr. D | 11/8 | 5.22 | 7.31 | 12.6 | 120/80 | + | Hyal. | Normal | Norm. | 17.7 |

temperature returned to normal by crisis and two days later the renal function index was determined. This was found to be high (28.2), although the blood urea nitrogen at this time had decreased to 20.9. The urine showed the presence of albumin but no casts. On April 3, 1925, three months later, the renal function index was normal (13.6) and the blood urea nitrogen was also normal (11.5).

CASE 7219.—A female, age thirty-nine, was admitted, complaining of difficulty in urination and pain in the lumbar region following a "heavy cold," also a sore throat and cough. During childhood she had had scarlet fever and frequent attacks of sore throat. In 1918 she had influenza, and a year before admission she had her tonsils removed. For the past few years she suffered considerably from severe headaches and impairment of vision. The diagnosis of acute nephritis with pharyngitis and bronchitis was made. The patient had badly infected teeth, inflamed throat, tenderness over the left kidney region and an albuminuric neuroretinitis. The blood pressure was systolic 130, diastolic 80, and the urine showed the presence of albumin and casts.

Chemical blood examination on January 2, 1925, showed urea nitrogen 18.5, creatinine 1.0, uric acid 3.3, sugar 90. These findings do not indicate retention of the nitrogenous constituents of the blood. On January 12, the renal function index was found to be definitely increased (22.3), while the blood urea nitrogen was still normal (17.9). After several weeks the patient was discharged greatly improved. On March 12, the patient reported that she did not have any of the symptoms previously mentioned and the renal function index had returned to normal (12.5). The blood urea nitrogen was 10 mg. per 100 c.c.

CASE 7295.—A watchman, age fifty-nine, was admitted, complaining of pain along the course of the right sciatic nerve, of two weeks' duration. Previous history was that of a gonococcus infection eighteen years ago. The tonsils had been removed thirty years ago and all the teeth had been extracted four years ago.

On January 12, the blood urea nitrogen was 15.6 mg. and the renal function index was 23.7. The urine was repeatedly negative and the blood pressure normal. The patient was kept in bed and given a soft low protein diet. February 18, a second examination showed the blood urea nitrogen to be 12.5 mg. and the index 20.4. The patient was discharged greatly improved. On March 31 the blood urea nitrogen was 14.7 and the functional index still high (20.4), showing that according to the functional index, the patient still had some impairment of renal function, although the blood figure still remained normal.

CASE 7392.—A man, age twenty-nine, complained of pain in the chest, cough, fever, and headache of ten days' duration. He had also had several attacks of vomiting during this time. For the previous three years he had nocturia, and for the past four months,

TABLE II

CASES SHOWING INDEX BETWEEN 18 AND 40

| HIST. NO. | DATE | URINE | | BLOOD | | URINE | | DIAGNOSIS | DIET | INDEX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | U. N. | T. N. | UREA N. | PRESSURE | ALB. | CASTS | | | |
| 6986 | 12/12 | 5.5 | 7.6 | 14.2 | 110/66 | 0 | 0 | Acute Rheumatic Fever | Soft | 19.5 |
| 6726 | 1/6 | 6.1 | 9.8 | 20.8 | 104/66 | + | Hyal. | Ac. Rheum. Fever | Soft | 33.4 |
| | 3/16 | 9.0 | 11.5 | 8.9 | 110/75 | 0 | 0 | | Norm. | 11.3 |
| Dr. J. | 1/7 | 10.6 | 14.3 | 16.6 | 130/85 | 0 | 0 | Normal | Norm. | 22.3 |
| 7244 | 1/10 | 5.1 | 6.9 | 20.9 | 130/90 | + | 0 | Lobar Pneumonia | Soft | 28.2 |
| | 4/3 | 9.6 | 11.5 | 11.5 | 120/80 | + | Hyal. | | Norm. | 13.6 |
| 7219 | 1/12 | 7.2 | 9.5 | 17.9 | 130/80 | + | Hyal. | Acute Nephritis | Soft | 22.3 |
| | 3/12 | 5.1 | 6.9 | 10.0 | 120/75 | 0 | 0 | | Norm. | 13.5 |
| 7295 | 1/12 | 7.5 | 11.4 | 15.6 | 124/64 | 0 | 0 | Neuritis | Soft | 23.7 |
| | 2/18 | 6.8 | 11.1 | 12.5 | 120/70 | 0 | 0 | | " | 20.4 |
| | 3/31 | 8.2 | 10.1 | 14.7 | | 0 | 0 | | Norm. | 18.1 |
| 9014 | 1/15 | 8.7 | 11.7 | 16.7 | 130/60 | 0 | 0 | Bronchopneumonia | Soft | 22.4 |
| 7284 | 1/15 | 5.3 | 7.5 | 11.7 | 110/70 | 0 | 0 | Chr. Myocarditis | Salt Free | 23.6 |
| 7392 | 1/25 | 9.4 | 10.6 | 35.7 | 162/104 | + | Hyal. Gran. | Grippe Ac. Neph. | Low Prot. | 39.4 |
| 7268 | 1/25 | 5.03 | 9.7 | 10.0 | 104/90 | + | Hyal. | Pylorospasm | Soft | 19.2 |
| 7413 | 1/27 | 3.8 | 4.2 | 36.0 | — | + | Hyal. Gran. | Acute Nephritis | Low Prot. | 39.5 |
| | 2/12 | 4.47 | 5.3 | 31.2 | — | + | Hyal. Gran. | " | " | 37.0 |
| | 2/19 | 3.9 | 4.0 | 12.5 | 110/80 | + | " | " | " | 12.8 |
| 3604 | 2/4 | 7.23 | 11.1 | 12.5 | 150/75 | + | Hyal. | Cirrhosis | Soft | 19.2 |
| 7736 | 2/26 | 6.0 | 9.4 | 18.0 | 155/100 | + | Hyal. | Chr. Myoc. Cirrhosis Chr. Myoc. | Soft | 28.2 |
| 7524 | 2/28 | 8.6 | 14.3 | 14.7 | 116/78 | +++ | Hyal. | Chr. Myoc. | Soft | 24.4 |
| 7745 | 3/4 | 14.1 | 15.6 | 16.6 | 120/75 | + | Hyal. | Peptic Ulcer. | Spec. Ulcer | 18.3 |
| 7729 | 3/6 | 6.3 | 8.0 | 15.6 | 135/85 | 0 | 0 | Pleurisy | Soft | 19.8 |
| 7516 | 3/9 | 7.5 | 9.6 | 17.8 | 145/95 | ++ | Hyal. | Hematuria Rt. Kidney | Soft | 22.6 |
| 7887 | 3/11 | 5.8 | 7.5 | 14.0 | 140/75 | ++ | Hyal. | Acute Nephritis | Mod. Karell | 18.1 |
| 7471 | 3/14 | 7.0 | 9.6 | 31.2 | 190/85 | + | Hyal. | Cardiac Nephritic | Karell | 22.7 |
| 7933 | 3/17 | 5.6 | 8.2 | 17.8 | 180/100 | ++ | Hyal. | Cardiac Decomp. | Karell | 26.0 |
| 7930 | 3/18 | 4.8 | 8.7 | 10.4 | 115/55 | +++ | Pus Cells | Calculus (Renal.) | Soft | 18.8 |
| 7989 | 3/23 | 5.1 | 10.0 | 10.0 | 150/15 | ++ | Hyal. | Chronic Endocard. | Soft | 19.6 |
| 7174 | 3/23 | 4.9 | 7.6 | 17.0 | 170/85 | +++ | Hyal. | Chronic Cardiac | Soft | 26.3 |
| 8019 | 3/27 | 5.3 | 9.6 | 11.4 | 90/40 | 0 | 0 | Diabetes | Spec. Diabetic | 20.6 |
| " | 4/6 | 4.6 | 6.0 | 9.2 | " | 0 | 0 | " | " | 12.0 |
| 8031 | 3/30 | 6.6 | 9.4 | 25.0 | 180/65 | + | Hyal. | Bronchopneumonia | Karell | 35.6 |
| 7804 | 3/30 | 8.2 | 12.0 | 17.0 | 165/90 | ++ | Hyal. | Chronic Nephritis | Soft | 24.8 |
| 7991 | 4/7 | 5.9 | 9.2 | 12.5 | 115/85 | ++ | Hyal. | Emphysema Chr. Myoc. | Soft | 20.1 |
| 8203 | 4/14 | 4.7 | 7.6 | 17.0 | 133/75 | + | Hyal. | Chronic Myocard. | Karell | 27.5 |
| 7876 | 4/25 | 4.5 | 6.4 | 13.2 | 120/75 | + | Hyal. & Gran. | Acute Nephritis | Liquid | 18.7 |
| 8305 | 4/24 | 4.8 | 6.2 | 16.6 | 130/75 | ++ | 0 | Impacted Feces | Soft | 24.2 |

TABLE III

INDEX ABOVE 40

| HIST. NO. | DATE | URINE | | BLOOD | | URINE | | DIAGNOSIS | DIET | INDEX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | U. N. | T. N. | UREA N. | PRESSURE | ALB. | CASTS | | | |
| 6601 | 11/3 | 1.49 | 2.41 | 125 | 156/88 | +++ | Gran. Hyal. | Chronic Nephritis, Uremia | Low Prot. | 202.1 |
| 6955 | 12/8 | 5.4 | 10.27 | 22.7 | 130/90 | + | 0 | Arterio-sclerosis | Soft | 43.2 |
| 7245 | 1/10 | 5.96 | 9.0 | 41.6 | 180/40 | + | Gran. Hyal. | Chr. Neph. Chr. Myoc. | Karell | 62.8 |
| 7730 | 4/4 | 6.7 | 13.1 | 19.0 | | + | Hyal. | Rheumatic Fever | Soft | 50.4 |
| 7392 | 4/9 | 4.9 | 6.9 | 62.5 | 205/125 | +++ | Gran. R. B. C. | Grippe Ac. Neph. | Karell | 88.0 |
| 7929 | 4/16 | 5.7 | 15.9 | 22.7 | 165/100 | +++ | Hyal. | Acute Nephritis | Karell | 63.3 |

pain in both kidney regions. Physical examination disclosed an acutely inflamed throat and fever. The diagnosis of influenza was made. Eye-ground examination showed a retinitis with tortuosity of the blood vessels, as seen in cases of hypertension. The blood pressure was systolic 205, diastolic 125. The urine showed the presence of albumin and hyaline casts.

On January 17, 1925, a chemical blood examination gave the following values: urea nitrogen 50 mg.; creatinine 1.4, uric acid 6.0, sugar 141, and carbon dioxide combining power of the blood plasma 48.1 volume per cent, per 100 c.c. January 21, the chemical blood examination was repeated with these results: urea nitrogen 45.6, creatinine 4.5, uric acid 4.9, sugar 119. The increase in the creatinine content pointed to kidney impairment. On January 25, the renal function index was found to be 39.4. The patient was put on a low protein diet and kept in bed. April 9, (see Table III) the index was again determined and found to have increased to 88.0. Clinically, the patient was much worse.

CASE 7413.—This case was an acute hemorrhagic nephritis in which the renal function index was determined at varying intervals. On January 27, the blood urea nitrogen was 36.0 and the index 39.5. The patient was placed on a low protein diet and on February 12, the index was 37.0. One week later the index was again determined and it was found to be 12.8. Clinically, the patient showed marked improvement, while his urine still contained albumin and casts.

CASE 8019.—A man, age twenty-eight years, was admitted on March 23, 1925, with diabetic coma. Patient was first told of his diabetic condition three years prior to present admission. Two years ago he had swollen legs which lasted several weeks. On March 27, four days after admission the renal function index was 20.6 with a blood urea nitrogen of 11.4. On March 30, slight puffiness of the face was noted and on the following day this had extended, causing marked swelling of the face and extremities. Under proper diet and rest in bed the edema cleared up. Urine examinations were at all times negative for albumin and casts. On April 6, a second renal function test was done and this time the index was 12.0.

Group 3 (Table III) cases showing an index above 40. There were six cases in this group, of which one was a repetition of a case included in Group 2. The urinary and blood findings and clinical course in all these cases are so definitely indicative of renal impairment, that it would hardly be worth while to enter into any further detailed discussion of them.

## DISCUSSION

Chemical examination of the blood is of great value in determining the retention of waste nitrogenous constituents. Such examination, however, gives no indication of the excretory capacity of the kidney. It has been the experience of all who have observed any considerable number of cases to

meet with instances in which the blood chemistry has been normal up to within a few hours previous to death, while the clinical evidence supported by postmortem examination, warranted a diagnosis of severe nephritis. Again the blood chemical examination may give results which are considered as high normal or only slightly increased above normal, and no definite statement can be made as to the presence or absence of a nephritis, or as to its severity.

As previously stated, the normal eliminating power of the kidney as regards the urea nitrogen is 80 to 90 per cent of the total nitrogen. This percentage elimination is diminished in nephritis, usually before definite changes in the blood nitrogenous constituents are obtained. An example of this decreased eliminating power of the kidney is encountered in mechanical obstruction, as in ligation of the ureters, and in acute conditions such as mercury, uranium or tartrate nephritis, where the decreased elimination is the first symptom, after which retention of the waste nitrogenous constituents of the blood results.

It would seem that any procedure involving a study of both the elimination of nitrogenous constituents and their concentration in the blood, would give a more accurate estimate of the degree of renal function. The present index offers such a combination of methods. The procedure has the advantage of being simple in technic, of imposing no hardship on the patient, and of lending itself to routine procedure. Experience indicates that the renal function index may be helpful in the diagnosis of doubtful cases of nephritis.

## SUMMARY

1. The index of Stander, Duncan and Moses has been studied in 60 cases.

2. The normal index has been found not to exceed 18.

3. An index between 18 and 40 is indicative of impairment of renal function, although the blood figures may be within normal limits.

4. Changes in the index in nephritis may be of aid in prognosis.

5. In severe cases of nephritis, indices higher than 40 have been obtained, one case reaching 202.

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# A STUDY OF THE ANTIGEN USED IN THE WASSERMANN TEST FOR SYPHILIS*

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## I. THE RÔLE OF THE ANTIGEN IN THE FIXATION OF COMPLEMENT AND IN THE FORMATION OF A PRECIPITATE

DESPITE the immense amount of work that has been done in the endeavor to find out how the antigen brings about complement fixation in the presence of syphilitic serum, the exact nature of the Wassermann reaction is still a mystery. One thing, however, is certain, that the reaction is not due to a specific antigen-antibody union. This view was strengthened by the findings of Schereschewsky[1] and Noguchi[2] that a specific antigen prepared from cultures of Spirochete pallida cannot be used as successfully in the diagnosis of syphilis as antigens prepared from tissues of normal organs.

A number of theories have been advanced to account for the Wassermann reaction.

Noguchi[3] demonstrated that the active antigenic principles in normal tissues were lipoids, and he concluded that the reaction depends upon lipotropic substances present in the patient's blood serum. He also claims[4] that there exists a certain relationship between the iodine number of an antigen and its binding power. This seems to indicate that a chemical union may take place at the free bonds of the unsaturated carbon atoms in the lipoid molecule with the substances present in the syphilitic serum, and a deviation of complement is brought about when this union has taken place.

Manwaring[5] advanced the opinion that there is a proteolytic ferment present in the guinea-pig's blood serum, which has the power to destroy complement. The fixation of complement is due to substances present both in the antigenic extracts and in the patient's serum, which bring about favorable conditions for the activation of the ferment.

Levaditi and Yamanouchi[6] believe that the reaction depends upon the union of two colloidal factors, one consisting of the lipoidal substances in the antigen, and the other present in the patient's blood. Both substances, they believe, are alcohol-soluble and nonprotein in nature.

Schmidt[7] considers the reaction colloidal in nature and believes that it depends upon the union of the colloids in the antigen with the globulins in the patient's serum. These globulins are increased in amount and changed in nature in syphilitic patients. No such union occurs in normal serum, it being prevented by the albumins which act as protective colloids.

Citron,[8] and Weil and Braun[9] forwarded somewhat similar theories, the so-called "auto-antibody" theories, both resting upon the belief that in the blood of syphilitic persons antibodies to the lipoids in the antigenic extracts

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exist; these antibodies being produced by the absorption of albumin-lipoid substances, elaborated by the growth of Treponema pallidum, and the subsequent production of antibodies against these substances.

Porges and Myer[10] were the first to observe that a precipitation occurred when a lecithin emulsion was added to syphilitic serum.

Many workers, therefore, are of the opinion that the Wassermann reaction is due primarily to the precipitation of globulins by the lipoidal colloids of the antigen, and that the resulting precipitate is capable of absorbing complement; thus the removal of complement is dependent upon the precipitate formation.

None of the theories, however, rest upon sufficient experimental evidence to be accepted as the true explanation of the process of complement fixation in syphilis.

Whether the deviation of complement actually depends upon the formation of a precipitate by the combination of the antigen with syphilitic serum has not really been demonstrated. Hull and Faught[11] attempted to separate the precipitate formed in the "Sachs and Georgi" test[12] and to determine whether it is entirely responsible for the Wassermann reaction. They centrifuged the precipitate at high speed for an hour and performed a complement-fixation test on both the precipitate and supernatant fluid. They obtained a positive Wassermann test with both the precipitate and the supernatant fluid, thus obtaining inconclusive results.

Dean[13] found that the proportion of antigen and antibody favorable for rapid and complete precipitation does not favor the most complete complement fixation.

In a previous paper,[14] I have shown that the antigenic substances in beef heart are thermostabile and can withstand boiling or even heating in the autoclave under 15 pounds pressure for an hour without impairing their antigenic properties. Accordingly, I have described a method for preparing an antigen by using high temperatures. The antigen thus prepared gave good results when used in the Wassermann test. When I attempted to use this antigen in the precipitin test according to the method of Sachs and Georgi, however, I obtained negative results. The explanation for this was that the precipitating property of the antigen was destroyed by the heat employed in the preparation of the antigen. To prove that such was the case, two identical antigens were prepared, except that one was made at room temperature while the other was heated. The results were, as expected, that the antigen prepared at low temperature gave heavy precipitates with positive syphilitic sera, while the antigen prepared at high temperature gave no precipitates whatever.

Thus it seems that complement fixation does not depend upon the formation of a precipitate; the two phenomena do not run parallel courses, but they probably represent different phases of the same phenomenon. Yet, one cannot accept this as truth with any degree of certainty, for failure to notice a precipitate macroscopically does not prove the absence of an invisible precipitate: we know that albumin particles may aggregate into larger particles without causing precipitation, provided the excess of one of the precipitate-forming colloids acts as a protective colloid.[15] Thus, further evidence is necessary to settle this question.

## II. THE LIPOIDAL NATURE OF THE ANTIGEN

A question of great importance is whether the antigen is a pure lipoid or a lipoid-protein combination. According to Landsteiner,[16] mere solubility of a substance in organic solvents cannot be taken as proof of its lipoidal character. It is very probable that nonlipoidal substances may go into solution in organic solvents when lecithin is present. Michaelis and Rona[17] have shown that alcoholic lecithin solutions protect albumoses from the precipitating action of alcohol. Müller[18] found that the antigen of typhoid bacilli is soluble in chloroform, in the presence of old preparations of lecithin. Thus, there is a possibility that the antigenic property of the alcoholic extracts used for complement fixation in the Wassermann test is due to a lipoid-protein combination which permits the presence of protein, mechanically or chemically united to the lipoid in the alcoholic solution.

Since mere extracting with fat solvents does not separate all the protein from the lipoids, other means have to be applied for removing all traces of protein from lipoids. To accomplish this I have digested beef heart muscle with trypsin previous to extraction with alcohol. Trypsin was chosen in preference to pepsin because its digestive power is more complete, reducing protein to amino acids, and also because it works in a neutral medium.

*Procedure.*—Trypsin was prepared according to the method of Stutzer and Merres,[19] which is as follows:

A calf pancreas was freed from fat, ground through a meat grinder, and crushed with sand in a mortar. This was left in the mortar for twenty-four hours (during this period the trypsinogen is converted into trypsin). Water was then made alkaline with lime, and three parts added to one part of pancreas (without the sand) and one part glycerine. The mixture was allowed to stand for four days. The mass was then squeezed through flannel, filtered through filter paper and evaporated to dryness in a vacuum.

The reaction of the trypsin was neutral to litmus. The lime was added to saponify fat and to destroy lipase. (Too much lime weakens the tryptic activity.)

Fifty grams of beef heart were ground up and divided in two equal parts. To one portion was added 100 c.c. of water and 0.25 grams of trypsin. This was digested for six hours on the water-bath at 38° C. The tissue dissolved and gave no biuret test. It was then evaporated to dryness on the water-bath. The ether-soluble substances were now removed by extracting with ether in a Soxhlet apparatus, and the residue was extracted for two hours with 25 c.c. of hot alcohol.

The other portion of the beef heart was dried on the water-bath, extracted with ether, and the residue extracted for two hours with 25 c.c. of hot alcohol; thus the two antigens were prepared identically with the exception of the tryptic digestion, the last one serving as a control.

*Titration of Antigens.*—The antigens were titrated with an antisheep hemolytic system, one-fourth Wassermann quantities being used. The complement was titrated immediately before use. Two units of complement and two of amboceptor were used. Complement fixation was done in the ice box for four

hours; sensitized red cells were then added and the second incubation was done in the water-bath at 37° C. for twenty minutes and the results read.

The antigens were made up in two dilutions: 1 to 10, and 1 to 40, by slowly adding saline from a pipette to a measured amount of antigen into a flask, shaking it gently all the while.

A series of 13 tubes were set up, the first four tubes receiving increasing amounts of the 1 to 40 diluted antigen, and the remaining tubes receiving increasing amounts of the 1 to 10 diluted antigen as shown in Table I.

TABLE I

TITRATION OF ANTIGENS

| | BINDING POWER | | | | | | ANTICOMP. PROPERTY | | | | | HEMOLYTIC POWER | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TUBE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 1/40 Ant. c.c. | .01 | .02 | .05 | 0.1 | | | | | | | | | |
| 1/10 Ant. c.c. | | | | | .05 | 0.1 | 0.2 | 0.5 | .75 | 1.0 | 1.2 | 0.5 | 1.0 |
| Final dilution | 1/4000 | 1/2000 | 1/800 | 1/400 | 1/200 | 1/100 | 1/50 | 1/20 | 1/13 | 1/10 | 1/8 | 1/20 | 1/10 |

The first six tubes contain antigen in dilutions ranging from 1-4000 to 1-100; they all contain 0.05 c.c. positive serum each, thus serving as a test for the antigenic power of the antigens.

Tubes 7 to 11 inclusive contain no serum (except tube 7) and serve to demonstrate the anticomplementary property.

Tube 7 contains 0.1 c.c. of negative serum and serves to test the specificity of the antigens.

The last two tubes contain no serum and no complement, and show the hemolytic power of the antigens.

The following signs were used in writing the results: ++++ = complete inhibition of hemolysis; +++ = slight hemolysis; ++ = half inhibition; + = slight inhibition; 0 = complete hemolysis.

TABLE II

RESULT OF ANTIGEN TITRATIONS

| ANT. DILUTION | 1-40 | | | | | | 1-10 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PROPERTIES | ANTIGENIC | | | | | | ANTICOMP. | | | | | HEMOLYTIC | |
| TUBES | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Ant. c.c. | .01 | .02 | .05 | 0.1 | .05 | 0.1 | 0.2 | 0.5 | .75 | 1.0 | 1.2 | 0.5 | 1.0 |
| Trypsin digested | 0 | 0 | ++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | + | ++ | ++++ | ++ |
| Control | 0 | 0 | +++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | ++ | +++ | ++++ | ++++ |

As shown in Table II the digestion of the protein did not destroy the antigenic properties of the beef heart, thus indicating that the active principle in the antigen used in the Wassermann test for syphilis is not a protein nor a protein-lipoid combination, but a pure lipoid.

## SUMMARY

1. Heating of the beef heart tissue or of the alcoholic extracts used as antigens in the Wassermann test for syphilis does not affect their complement-fixation power but does destroy their precipitate-forming property. Whether

this shows that the precipitate formation and complement fixation are two distinct and separate phenomena cannot be told, for failure to see a precipitate macroscopically does not prove the absence of an invisible precipitate.

2. The digestion of beef heart muscle with trypsin in neutral solution does not injure the antigen; hence the antigenic substances are true lipoids and not combinations of lipoids with proteins.

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## PROGNOSTIC VALUE OF EOSINOPHILES IN CASES OF EAR INFECTION*

By Viola Brewer, Ph.G., B.S., Spokane, Wash.

MUCH has been said as to the value of the leucocyte and differential count as an aid to prognosis, but I have seen very little concerning the value of the eosinophiles.

In our general hospital laboratory, we have been called upon to make a leucocyte and differential count daily for several days, and sometimes for weeks, for patients suffering from acute or chronic ear infections. It has been noticed that when the infection is subsiding the eosinophiles appear in the blood stream, together with a lower leucocyte count. The temperature also becomes lower at the same time.

I have made a study of fifty hospitalized cases of this nature for whom two or more leucocyte and differential counts were made. Of these counts, 130 showed eosinophiles and 181 did not show any. The following work has been based upon the 130 differential counts which showed eosinophiles.

As observed from Chart I, the greatest percentage of eosinophiles occurs when the temperature is between 98° and 100°, i.e., near normal. In those cases where a temperature of 104° to 106° was attained, however, the eosino-

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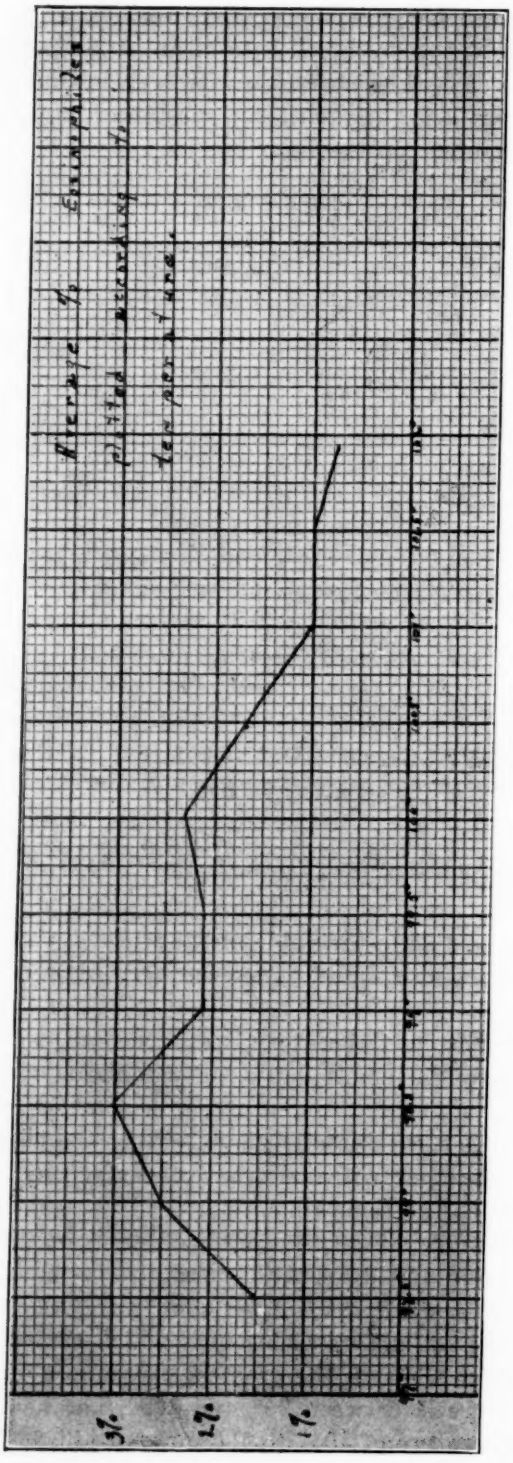

Chart I.

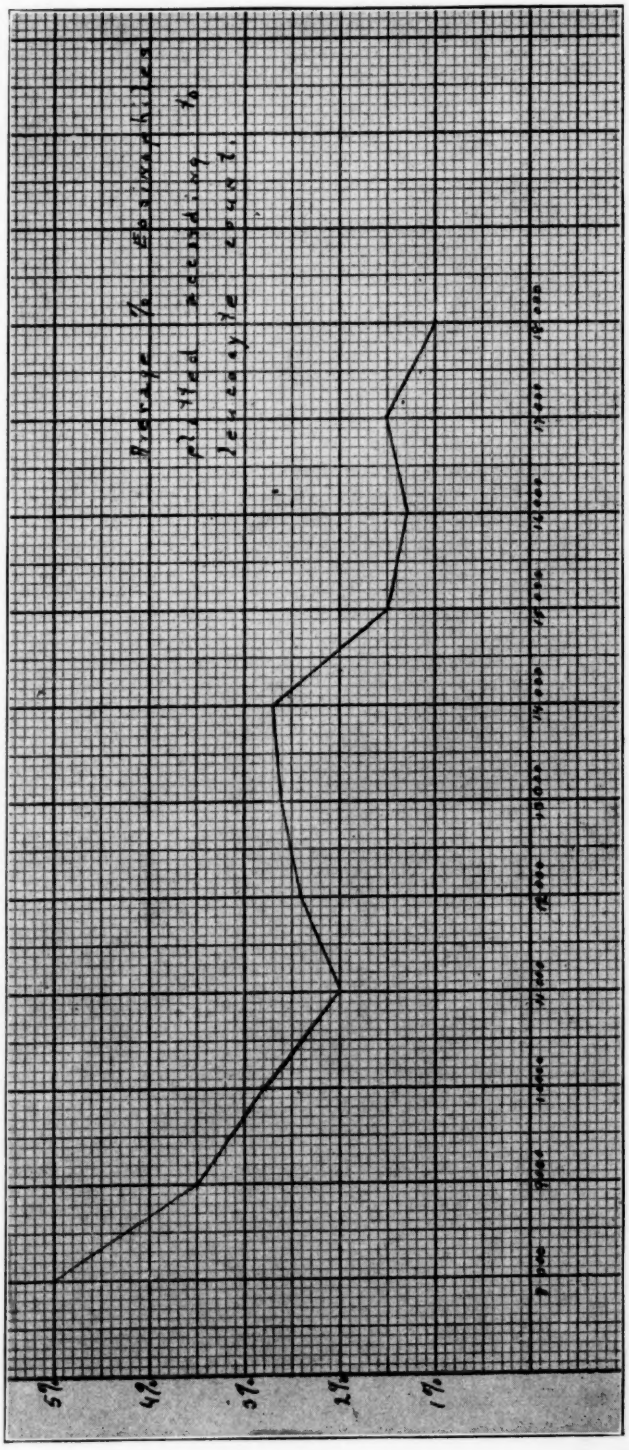
Average % Eosinophiles
of Total Secretion
during the
first week
5%
4%
3%
2%
1%

Chart II.

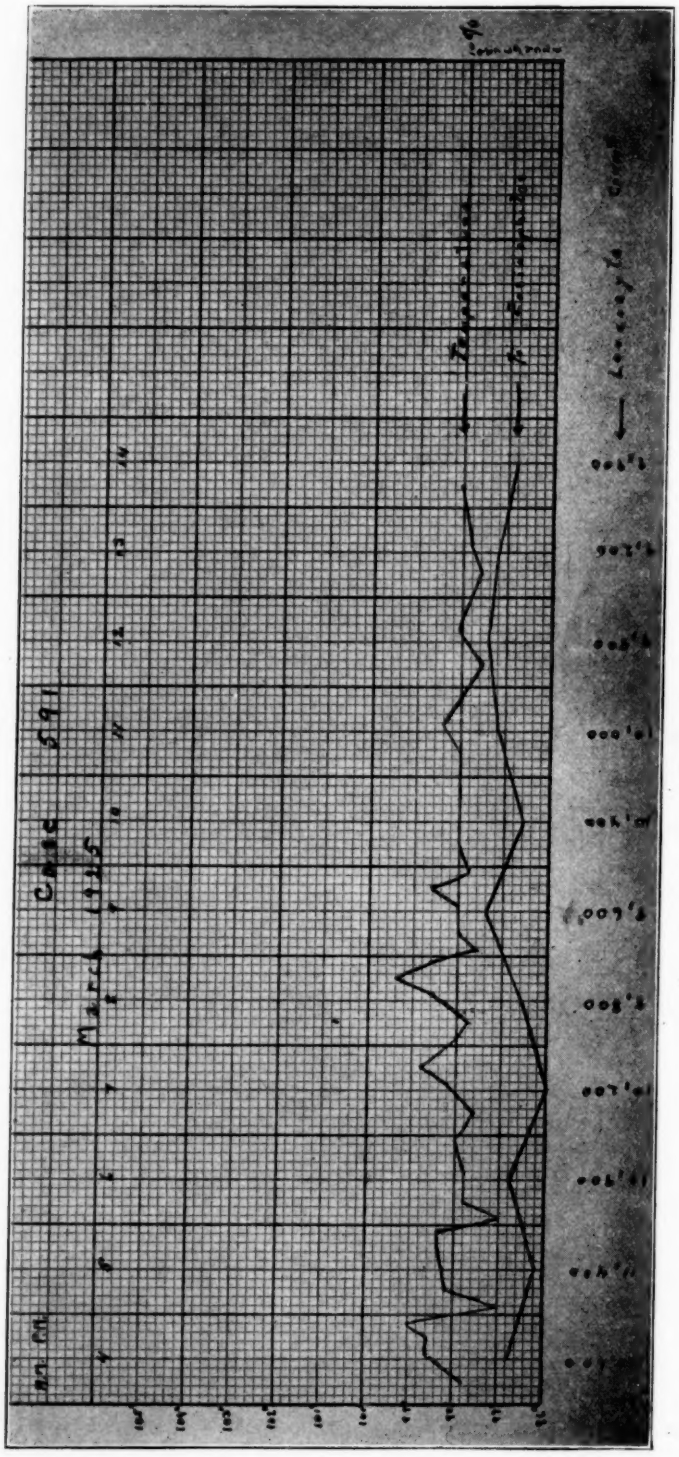

Chart III.

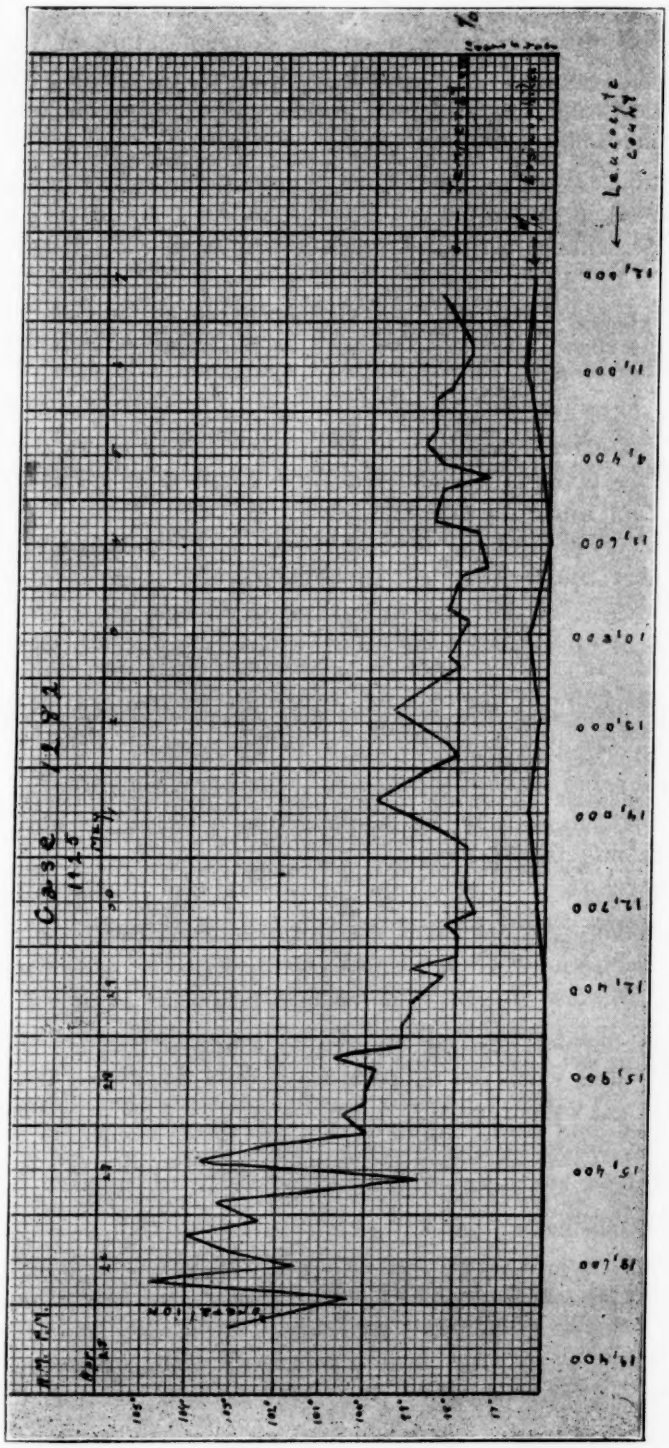
Case 1182
Temperature
Leucocyte count
Eosinophiles
Operation
12,600
11,000
4,900
13,600
10,800
13,000
14,000
12,700
12,400
15,900
15,400
18,600
19,400

Chart IV.

philes appeared almost simultaneously with a decline in temperature and a subsidence of the clinical symptoms, so, in a few cases, we found 1 per cent or 2 per cent of eosinophiles accompanying a temperature of 103° to 104°. These were cases in which the clinical symptoms also indicated improvement. In no case did eosinophiles occur just previous to a relapse or when the temperature was markedly subnormal.

Chart II illustrates the curve when the eosinophiles are considered with relation to the leucocyte count. We find that the same is, in general, true. The greatest percentage of eosinophiles occurs when the leucocyte count is between 8,000 and 14,000, although, in some cases that had been running a very high count, 1 per cent or 2 per cent occurred as high as 25,000. Here too, the patient showed an improvement in temperature and other clinical symptoms.

The highest per cent of eosinophiles recorded was 10 per cent in a chronic case, eight hours after drainage was established. As long as there was free drainage and the temperature and leucocyte count were near normal, 3 per cent to 6 per cent was quite common.

Of the 50 cases studied, 19 underwent mastoid and 9, myringotomy operations. The occurrence of eosinophiles was the same whether an operation was performed or the patient recovered without an operation. They appeared when the patient was convalescing and disappeared when there was a relapse.

Chart III illustrates the curve of temperature and eosinophiles, with leucocyte count noted, in the typical case of a man with acute purulent otitis media who recovered without submitting to an operation.

Chart IV is that of a boy who underwent a simple mastoidectomy and recovered with no complications.

Both of these cases were selected at random and illustrate the fact that eosinophiles appear in the differential count when the patient's condition is improving.

## CONCLUSIONS

1. Eosinophiles occur in the differential count of patients suffering from ear infections: (a) when the temperature is on a downward trend and nearing normal but not subnormal, (b) when the leucocyte count is tending toward normal and the clinical indications are favorable.

2. Eosinophiles are a favorable prognostic sign when taken into consideration with the leucocyte count and the clinical symptoms.

# THE ANTISCORBUTIC VITAMINE IN FRESH BEEF*

By Grace Medes, Minneapolis, Minn.

IN VIEW of the repeated statements that fresh meat will prevent scurvy in man,[1] various attempts have been made to test the assertion by experiments with guinea pigs. Chick, Hume and Skelton[2] obtained no protection when they fed guinea pigs with 10 c.c. of fresh raw beef juice daily. Pitz[3] believed that death of guinea pigs was delayed by feeding dried meat, but Dutcher, Pierson and Biester,[1] Chick, Hume and Skelton,[2] and Givens and McCluggage,[4] ascribe the delay of death in his experiments to the fresh milk which was supplied in unlimited amounts. Dutcher, Pierson and Biester, in the same article,[1] show that aqueous extracts of fresh beef do not prevent scurvy. In another instance they fed 5 grams of chopped raw beef daily, with similar result. Givens and McCluggage[4] fed 1.5 grams of meat dehydrated *in vacuo* at a temperature not exceeding 65° for a period of twelve hours, and found that the onset of scurvy was not delayed.

The experiments quoted above seem incomplete on two points. First, in the case of meat extracts or expressed juice, it may be that the vitamine is retained in the meat residue. Second, in those instances in which fresh meat itself was fed, it is possible that the amount consumed daily was too slight to afford protection to an animal requiring as large an amount of the antiscorbutic factor as the guinea pig.

The first series of experiments was begun during the summer of 1920 and was previously published.[5] It was hoped that the guinea pigs could be induced to eat fresh meat in larger amounts than they had in any of the previous experiments. Fresh beef was secured daily from a meat market and was kept on ice until time of feeding. The animals were fed three times daily and at each feeding the meat was freshly scraped or ground in a meat chopper, as it was found that after it had been ground for a few hours the guinea pigs would not touch it. The ground beef was then stirred with a small amount of alfalfa meal and wheat flour, which had been previously mixed in equal parts by weight. The whole was then made into a paste-like ball with water, and rolled in alfalfa meal. Each animal was given daily 2 grams of milk powder stirred with water. Young guinea pigs were selected, ranging in weight from 100 to 250 grams in weight, as the younger animals could be trained more successfully to eat the beef. By observing these precautions, the guinea pigs were induced to consume an average of 15 to 30 grams of fresh meat daily. The alfalfa meal, milk powder and flour were tested on guinea pigs, and at that time were declared to be devoid of vitamine *C*.

*From the Physiological Laboratories of the University of Minnesota Medical School and Vassar College.

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Twelve guinea pigs were used in the experiment. Two served as controls, one of which died of scurvy on the thirty-fifth day and the other of which was killed and autopsied on the twenty-sixth day. Both showed lesions of scurvy. Three refused to eat the meat and died, probably of starvation, after about a week. Five guinea pigs on the meat diet died of scurvy on the twenty-third, twenty-fourth, twenty-fifth, and twenty-sixth days, respectively. Two received orange juice with their food after the twenty-fifth day when they seemed to be dying of scurvy. Both recovered completely and remained healthy on the meat diet plus orange juice, showing that the meat diet is not, in itself injurious to guinea pigs. As may be seen, no protection was afforded.

The following winter the experiment was repeated, modified in several respects. In the experiment described above, the control animals received alfalfa meal in larger amounts than those fed on meat. Since they outlived the latter, the question arose as to the possibility that the alfalfa meal contained traces of the antiscorbutic vitamine. In the following experiment, the alfalfa meal was weighed daily and the three animals received equal amounts. The milk powder was heated in an oven at 95° C. for two hours, to permit oxidation of any vitamine *C* which might possibly be present, and was weighed out in equal amounts to each guinea pig. Four healthy young animals were chosen, three of them from the same litter, and the fourth of the same age and approximately the same weight. The following diets were employed:

*A*. Alfalfa meal + wheat flour, equal parts by weight, + 3 grams milk powder daily. Ground oats supplied freely.

*B* and *C*. Alfalfa meal + wheat flour, equal parts by weight, in the same amount as supplied to *A*. Three grams milk powder daily. About 20 to 30 grams lean beef daily.

*D*. Cracked oats in unlimited amounts. Three grams milk powder daily.

*A* and *B* were each given, in addition, about 10 grams of lactose daily, dissolved in water, and fed by pipette at each meal. McClendon[6] has shown that by feeding lactose the intestinal contents of rabbits may be changed from slightly alkaline to slightly acid, and in a later experiment (private communication) he obtained the same result with guinea pigs. The possibility exists that there is some relation between the large amount of vitamine required by the guinea pig and the alkalinity of its intestinal content.

Lactose, itself, does not possess any antiscorbutic value, as has been shown by Cohen and Mendel[7] and by Hart, Steenbock and Smith.[8] They explain its action in delaying the appearance of scurvy in an experiment by Pitz,[9] by suggesting that it may have carried some vitamine as an impurity. In the present experiment, the lactose was dissolved in water and boiled for six hours to destroy any vitamine that may have been present.

The weights and appearance of symptoms of scurvy of the guinea pigs are given in Table I. Both animals receiving meat outlived the controls, *B*, the one which was given lactose in addition, surviving *A* by seven days and

TABLE I

WEIGHTS OF GUINEA PIGS
(*S* = SCURVY)

| DAYS | A | B | C | D |
|---|---|---|---|---|
| 1 | 215 | 216 | 219 | 210 |
| 6 | 221 | 230 | 235 | 220 |
| 11 | 245 | 221 | 227 | 250 |
| 15 | 237*S* | 233 | 243 | 267 |
| 16 | 240 | 244 | 244*S* | 275 |
| 17 | 240 | 249 | 243 | 273*S* |
| 22 | 238 | 256*S* | 237 | 242 |
| 26 | 223 | 249 | 239 | 212 |
| 27 | 220 | 247 | 235 | 214 |
| 28 | 204 | 247 | 235 | 191 |
| 29 | 192 | 237 | 235 | |
| 30 | 170 | 232 | 232 | |
| 31 | | 238 | 227 | |
| 32 | | 235 | 214 | |
| 33 | | 238 | | |
| 34 | | 230 | | |
| 35 | | 227 | | |
| 36 | | 224 | | |
| 37 | | 221 | | |

surviving *D* by nine days. *C* survived *A* by only two days. *B* first showed symptoms of scurvy seven days later than *A*.

## CONCLUSION

Meat may be fed to guinea pigs in considerable amounts, 20 to 30 grams daily, provided it is used fresh, and the guinea pigs are put on the diet when sufficiently young.

It possesses slight antiscorbutic value (sufficient to delay scurvy seven days). This has been shown by feeding it in combination with vitamine-free lactose which increases slightly the H-ion concentration of the intestine and thus may affect the stability of the vitamine present in the meat. This latter explanation is offered only as a suggestion.

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# THE INFLUENCE OF FOCAL INFECTIONS IN DIABETES AS SHOWN BY ALTERATIONS OF THE BLOOD-SUGAR CURVE*

By Blair Holcomb, M.D., Portland, Ore.

THIS is a report of the histories of two patients with mild diabetes, which illustrates the effect of focal infection on the glucose tolerance and rather supports the theory of the infectious cause of diabetes.

Medical literature contains no reference to comparative blood-sugar curves before and after the removal of foci of infection in diabetic individuals. The lowering of tolerance by acute infections, such as measles, furunculosis, dental abscesses, etc., in an established case of diabetes is well known. Sansum[1] noted the decrease in tolerance in a normal person with acute tonsillitis. The rôle which influenza may have played in producing degenerative islet changes cannot be proved, but clinical evidence points strongly toward it as one of the major infectious causes following the late epidemic. Working with a large series of arthritides, Pemberton,[2] using the Janney[3] glucose meal, noted sustained elevations of the blood sugar in active joint infections and an abrupt return to a normal response following the removal of focal infections. Lusk,[4] quoting Rosenthal, states that diphtheria toxin given to rabbits renders the liver incapable of retaining glycogen and a hyperglycemia occurs. Olmstead[5] was unable to duplicate in arthritis the high curves of Pemberton, except with those patients who were febrile. Complete recovery of glucose tolerance, proved by blood-sugar curves, has not been reported, though Rohdenburg's[6] case of spontaneous disappearance of glycosuria at the menopause in a previously proved diabetic patient, showed hypertrophic pancreatic islets and an atrophy of the thyroid. The influence of infection was not considered nor were blood-sugar curves determined.

The mechanism by which toxins may influence the height of the blood-sugar curve is outlined by Olmstead and Gay.[7] They state in part: "1. Toxins may directly stimulate the action of liver and muscle diastase or inhibit the glycogenic power, or possibly disturb the hepatic acid base balance. 2. Focal toxins may act on glycogenolysis through their effect on the suprarenal medulla directly or through the autonomic reflex. 3. Focal toxins may also act on higher cerebral centers." With the discovery of the specific substance, insulin, the last link in the chain of evidence is complete. One may add, then, that most important of all may be the inhibitive effect of focal toxins or bacteria on the islands of Langerhans.

Mr. S., aged forty-two years, an American merchant, was seen June 5, 1923, because of a glycosuria that was discovered one week previously. Polyuria, polydipsia, and weakness began three months before, and a total loss of fifteen pounds of weight had occurred. His

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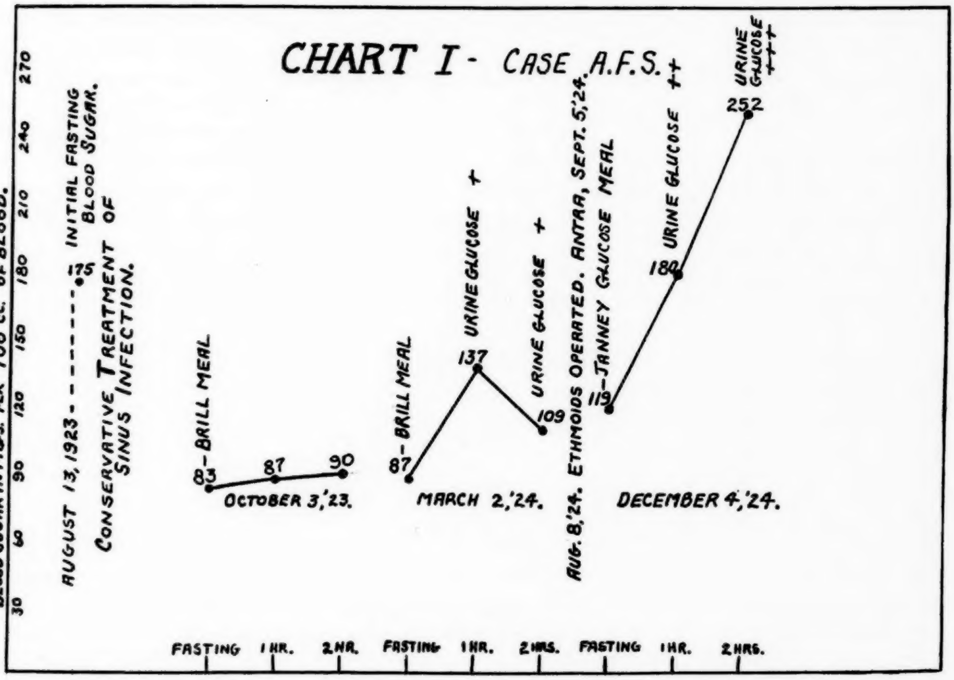
past history was negative except for jaundice with fever, pain, nausea and vomiting in 1908, but with no subsequent symptoms of gall bladder disease, scarlet fever as a child, and much nose trouble extending back for at least ten years. The latter consisted of mucopus drainage into the nasopharynx, many colds and finally an almost total obstruction of the nasal passages, so that at four different times during the ten years polyps had been removed, the last time in 1920. Physical examination showed an undernourished man 5 feet, 9 inches tall, weighing 138 pounds. The positive physical findings were one devitalized tooth, crepitant and mucous râles in both bases of the lungs, moderately enlarged thyroid (small colloid adenoma), and a blood pressure of 135-88. Nasal examination showed a bilateral hyperplastic ethmoiditis and purulent antra. Glucose was found in both morning and evening urines. The white blood cells numbered 13,500; the differential count, red blood cells and hemoglobin were normal. The stomach chemistry showed 12 free acid and 20 total acidity. The blood



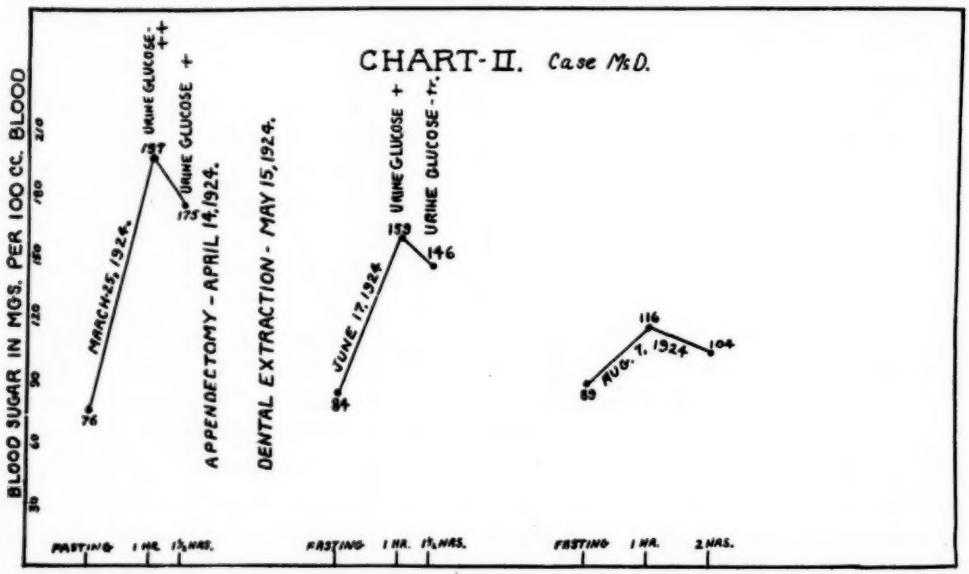

Wassermann was negative. Gastrointestinal roentgenologic study gave no evidence of abnormality. Stereoscopic chest films revealed a rather pronounced bilateral peribronchial thickening and increased hilus shadows. The fasting blood sugar was 175 mg. per 100 c.c. of blood. A diagnosis of diabetes mellitus, chronic sinusitis and bronchitis was made and the patient was placed in the hospital. On a 91-G* the twenty-four-hour urine was negative the following day to Benedict's reagent, and the day following the blood sugar was 104 mg. The diet was rapidly increased to a G of 125, following which the blood sugar remained 104 mg. Subsequent diet adjustment carried the patient's G to 172, which raised his blood sugar to 117 mg. Reference to Chart I will show the details of the blood-sugar curves.

During this time the patient's antra had been washed several times. His cough and sputum had stopped, he had gained in weight to 141 pounds, and felt very well. He went home on June 29 on a diet G of 172 gm. On July 6 his blood sugar was 92 mg., and both antra were clear. On August 14 the blood sugar being 83 mg., his G was raised to 218.

*G refers to grams of glucose in the diet and is calculated according to Woodyatt[8] from 100 per cent of the carbohydrate, 58 per cent of the protein and 10 per cent of the fat. The fatty acid glucose ratio of these diets, with the exceptions of diets above a G of 150 is 1.4. A diet of 1500 calories contains 91 G; 2000 calories 125 G; 2600 calories 150 G. Diets higher than these have an FA/G of about 1.1.

September 1 his blood sugar had dropped to 72 mg. On October 3, using the Brill[9] test breakfast, a blood-sugar curve was determined. The fasting blood sugar was 83 mg.; at one hour, 87 mg.; and two hours, 90 mg. We considered this a normal curve and instructed the patient to go onto a normal diet, restricting him only in the use of sugar. October 8 his fasting blood sugar was 92 mg. Reference to Chart I will show the details of the blood-sugar curves. On March 2, 1924, the blood-sugar curve was 87 mg. fasting; 137 mg. at one hour, and 109 mg. at two hours. Urine glucose by Benedict's reagent was present at the two latter times. On the basis of this low threshold alone, a diagnosis of renal glycosuria might have been justifiable, but knowing the previous history, it was untenable.

He did not report again until March 13, 1924. At this time, despite his statement that sugar had been found in the urine during the past three weeks, his fasting blood sugar was 82 mg. He was recovering from an acute cold of six weeks' duration during which his nasal discharge had increased. His diet was again restricted to a G of 250. The sinus infection was still active and subsequent conservative treatment was without results. The ethmoids




were operated upon August 8 and the antra later. Following this the antra were repeatedly clean and the nasal secretions were much decreased.

We did not see the patient again until December, 1924. Using the Janney glucose meal and giving 1.7 gm. glucose per kg. of body weight, the following curve resulted: fasting, 119 mg.; one hour, 180 mg.; two hours, 252 mg.; with sugar in the urine at one and two hours. Aside from the initial blood sugar the last fasting sugar was the highest he had ever shown. The diabetes is still of an essentially mild type, the patient's urine remains sugar free on a G of 250, and he holds a weight of 156 pounds and feels very well. Occasionally he experimentally raises his G to 275. This always causes glycosuria. The G of the Brill meal averages about 120 grams. This would amount to 360 grams for three meals. The first blood-sugar curve showed a definite normal response to this, so it is evident that he metabolized at least 360 grams at that time. Subsequently, despite the radical removal of pus, a loss of tolerance occurred.

Mr. McD., aged forty-three years, an American grocer, was seen March 18, 1924. Glycosuria was discovered during the course of a general examination. He had been suffering from cough, pain in the chest, in the back through the right sacroiliac region and down the right leg, and from polyuria for ten days following the removal of several abscessed teeth. He had previously had periodic attacks of pain in the right side of the abdomen for eight to

ten years, and pneumonia five years before with influenza. There was no history of diabetes in the family, no obesity, and no overeating. His weight was 187 pounds and height 5 feet, 8½ inches.

The physical findings were negative except for marked dental sepsis and tenderness along the cecum. The leucocyte count was 12,250 with 70 per cent polymorphonuclear neutrophiles. The blood Wassermann was negative. Both morning and evening samples of urine contained glucose with Benedict's reagent. The roentgenologic study of the gastrointestinal tract revealed a tender appendix with a broken lumen.

A blood-sugar curve, on March 25, using the Brill breakfast, showed a fasting level of 76 mg.; 197 mg. at one hour, and 175 mg. and one and one-half hours, with glucose in measurable amounts in the two latter urines. (Reference to Chart II will give the details.) The urine was rapidly desugarized on a G of 91 grams. The patient was then quickly raised to a G of 151 grams, on which his fasting blood-sugar level was 82 mg. On April 14 his appendix was removed. It was thick-walled, and the tip was swollen and acutely inflamed. After an uneventful recovery his blood sugar was 89 mg. fasting. May 15 all devitalized teeth were extracted. June 17 his blood-sugar curve had lowered to 84 mg. fasting; 159 mg. at one hour, and 146 mg. at two hours, with a measurable amount of urine glucose at one hour and a trace at one and one-half hours.

Because the patient wished to return to his work in the woods, where a measured diet would be impossible, an attempt was made to measure his tolerance. His G was raised to 198 on June 19. The blood sugar two weeks later was 92 mg. The G was raised subsequently by adding white bread and potatoes, until on August 5 it was 300 grams. The blood sugar following the raise in diet was 104 mg. fasting. A third blood-sugar curve on August 7 was 89 mg. fasting; 116 mg. at one hour, and 104 mg. at two hours, with no sugar in the urine.

An opinion was given that the tolerance had reached practically the normal limit; the patient was told to return to a normal diet, and was only moderately restricted in the use of sugar. Three months later the patient reported by letter that his blood sugar was 106 mg. fasting.

## DISCUSSION

The above histories are significant because no evidence of obesity, overeating, or record of previous diabetes in the families could be obtained. They are further significant in that in both there was a clear-cut history of frank infection preceding the onset of diabetic symptoms. In Mr. S. the sinus infection had existed for at least ten years and there had been no acute exacerbation preceding the glycosuria, though later an acute cold had definitely lowered the tolerance. Washing the antra always had the opposite effect. Operating upon the ethmoids and antra did not raise the tolerance, but unquestionably removed the cause and cured the bronchitis. One might speculate that, had the nasal infection not been controlled, a more rapid progressive loss of glucose tolerance might have resulted. The final blood-sugar curve in this case shows an obvious diabetic range.

The removal of dental foci of infection is so definitely associated with the onset of diabetic symptoms in Mr. McD. that its influence in lowering the glucose tolerance cannot be denied. The effect of the appendectomy and the further dental extraction on the tolerance increase is debatable, as one frequently notes an almost unbelievable increase in the amount of glucose metabolized in mild diabetes by simply keeping the diet G well within the tolerance limit. On the contrary, many so-called refractory cases have yielded to the removal of focal infection. On the basis alone of the final blood-sugar

curve complete recovery of tolerance has apparently occurred. The objection may be raised, however, that because the Brill meal contains food which is absorbed over a longer time than the Janney glucose meal that it is not a sufficiently delicate test for mild diabetes. Brill, however, reported practically parallel curves in comparing the Janney meal with his breakfast in normal people and in mild diabetics.

(The blood-sugar methods used in this work were first, the Shaffer-Hartman[10] and later the Haskins-Holbrook[11] modification of the same.)

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# *LABORATORY METHODS*

## A GLASS DISC AS A STANDARD FOR THE DETERMINATION OF THE ICTERUS INDEX OF BLOOD SERUM*

By Marjorie Preston, A.B.

THE determination of the icterus index of the blood serum has been shown by Bernheim[1] and by Shattuck, Browne and Preston[2] to be of practical value to the clinician in ascertaining the accumulation of bilirubin in the blood.

The technic of the test, as described by Bernheim[1] is as follows: About 5 c.c. of blood are withdrawn from an arm vein and allowed to clot. After standing for a sufficient length of time, the blood is centrifuged until the serum is absolutely clear. Care must be taken to avoid hemolysis. The clear serum is then matched against a 1:10,000 solution of potassium dichromate (0.1 grams in 1,000 c.c. distilled water) which has been arbitrarily chosen as a standard solution. In our determinations the biocolorimeter,[3] an improved colorimeter of the plunger type has been used. The standard solution is placed in the left hand cup which is set at 15 mm. The serum is then placed in the right hand cup and matched against the standard. The scale reading is noted. The depth of the standard, divided by the scale reading of the unknown serum, gives the icterus index. For example: The scale reading of the serum is 3: the standard (15) divided by the scale reading of the serum (3) gives a quotient of 5, which is the icterus index.

In hyperbilirubinemia, the color of the serum may be too intense for a satisfactory comparison with the standard. In such cases the blood serum is diluted with physiologic salt solution (0.9 per cent sodium chloride solution) until it has roughly the same intensity of color as the standard. The unknown is then compared in the colorimeter with the standard. The icterus index is then corrected for the dilution of the serum. For example: if the blood serum were diluted ten times, and then read 3 against the standard at 15, the calculation would be $\frac{15}{3} \times 10 = 50$.

To obtain accurate and comparable results the potassium dichromate used should be Kahlbaum's *zur analyse* and should be weighed out on an analytic balance. The standard, when not in use, is kept in the dark and, to prevent a reduction to chromate, a few drops of concentrated sulphuric acid are added per 500 c.c. It was thought that the replacement of this standard by a glass disc would obviate the difficulties in the preparation and keeping

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of the dichromate standard. The Klett Manufacturing Company has prepared a disc of "Uran" glass which compares favorably with the potassium dichromate standard. The disc is 5 mm. in depth and is made with a flange which enables it to fit in the top of the plunger of the biocolorimeter. The plunger screws in as before but the cup is not used on the standard side. The determination is carried out exactly as described above except that the glass disc takes the place of the potassium dichromate standard solution. The color of the disc corresponds to the color of the standard solution set at 15 mm., making the calculation using the disc identical with that using the dichromate solution.

TABLE I

| | DISC | | DICHROMATE STANDARD | |
|---|---|---|---|---|
| PATIENT | READING | ICTERUS INDEX | READING | ICTERUS INDEX |
| De A. | 2.6 | 5.7 | 2.6 | 5.7 |
| A. T. | 2.2 | 6.8 | 2.2 | 6.8 |
| J. K. | 4.4–4.5 | 17.0–16.5 | 4.4 | 17.0 |
| F. P. | 4.6 | 16.3 | 4.6 | 16.3 |
| C. A. | 2.4 | 31.0 | 2.4 | 31.0 |
| F. P. | 1.8 | 8.3 | 1.8 | 8.3 |
| N. M. | 4.5–4.6 | 16.5–16.3 | 4.6 | 16.3 |
| E. G. | 3.2 | 4.7 | 3.2 | 4.7 |
| G. | 4.2–4.3 | 3.4–3.5 | 4.2 | 3.5 |
| C. A. | 1.5 | 50.0 | 1.5–1.4+ | 50.0–53.5 |
| F. M. | 3.5 | 4.2 | 3.3 | 4.5 |
| Y. K. | 6.2 | 12.1 | 6.1 | 12.2 |
| J. W. | 1.3 | 11.5 | 1.3 | 11.5 |
| J. R. | 0.9 | 16.6 | 0.9 | 16.6 |
| M. K. | 1.7 | 8.8 | 1.6 | 9.3 |
| S. M. | 1.0 | 15.0 | 1.0 | 15.0 |
| M. F. | 1.1 | 13.6 | 1.0 | 15.0 |
| J. G. | 5.2 | 144.0 | 5.2 | 144.0 |

In a series of 18 comparisons representing a wide range of indices, sera were matched with both the disc and the standard solution. The results are given in the accompanying table. It is seen that in 14 out of 18 comparisons, the readings with the disc and the potassium dichromate solution were identical. The other four show a variation of 0.1 in three of the cases and 0.2 in one. Although the match was not always perfect, the color of normal as well as pathologic sera varies to such an extent that a standard which would give a perfect match in every case would be impossible to find.

## SUMMARY

Nine months' experience with the disc standard in daily determinations of icterus indices has proved the disc to be as satisfactory as the dichromate standard for comparison with blood sera, and more convenient for use.

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# THE TECHNIC OF THE PATHOGEN-SELECTIVE METHOD OF CULTURE*

By Myer Solis-Cohen, A.B., M.D., and A. I. Rubenstone, M.D.,
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THE object of the pathogen-selective method of culture is to separate those microorganisms from the surface or interior of the patient's tissues which are probably pathogenic for him, and hence infecting him, from those which are probably nonpathogenic for him, of which he is merely a carrier. The attempted differentiation is made *in vitro,* by means of the patient's fresh, whole, coagulable blood, which is believed to possess bactericidal or bacteriostatic power against organisms that are nonpathogenic for him and to lack it against organisms that are pathogenic for him. This inference is based upon certain experimental work done on both men and animals.

## THE BACTERICIDAL OR BACTERIOSTATIC POWER OF WHOLE, COAGULABLE BLOOD

Wright[1] observed that blood as it issues from the capillaries has a bactericidal action upon pneumococci which is not found in defibrinated or citrated blood or in serum. Heist, Solomon Solis-Cohen and Myer Solis-Cohen,[2] seeking an explanation for natural immunity, found that if pneumococci are seeded *in vitro* in the fresh, whole, coagulable blood of the naturally susceptible mouse or rabbit, they multiply with great rapidity; while when similarly seeded in the blood of the naturally immune chicken or pigeon, they disappear. But they found that defibrinated chicken blood and pigeon serum exerted no bactericidal action upon pneumococci, this latter fact already having been shown by Strouse[3] and Kyes.[4] Lister[5] found that immunity to pneumococcus infection was not due to agglutinins and opsonins, while complement fixation has proved of little value in connection with the pneumococcus. Bull and Bartual,[6] using a different method of immunization from that employed by Heist and Solomon Solis-Cohen, found the whole, coagulable blood of the pigeon to inhibit the growth of pneumococci, which property was absent from mouse or rabbit blood, but stated that they found no proof of a true bactericidal action. Later (personal communication) Bull stated that he had been convinced that the whole, fresh blood of resistant animals may kill a certain number of pneumococci, having gained this impression from the fact that capillary tubes charged with high dilutions of culture and then filled with blood *failed to show growth after any length of incubation period,* but he still felt that it was impossible to say definitely whether this result is due to actual killing of the organisms or to an inhibi-

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tion of multiplication and spontaneous death. The real point of Heist and the Solis-Cohens' contention, however, and Bull now concedes it, is this: given a certain relation between the *number* of pneumococci (or other organisms) and the *volume* of blood, the organisms perish in the blood of the immune animal.

Smiley[7] found that the capillary tube (Heist-Lacy) test of Heist and the Solis-Cohens failed to distinguish between bactericidal and bacteriostatic power. He devised a test tube method which gave constant and conclusive results in indicating the bactericidal property of the blood of rabbits immunized against pneumococci, by which he demonstrated bactericidins in the blood of rabbits following the injection of pneumococci. The fact that the titer of bactericidal activity increases in proportion to the amount of pneumococci introduced suggests, according to Smiley, that there may be some relation between the bactericidal property of blood and active immunity.

Robertson and Sia,[8] who used a still different and quite elaborate method, state that their results indicate that the blood of resistant animals, at least of the dog and cat, possesses destructive properties for pneumococci, and that this destructive property is not possessed by the blood of certain susceptible animals. Their experiments suggest that natural immunity depends chiefly, if not entirely, upon this property. The leucocytes, they find, play an active part in this process, but whether or not the destruction of the pneumococci occurs entirely within the leucocytes is not determined. That the serum also plays a part is shown by the fact that when the serum of resistant animals was inactivated before being used in the serum-leucocyte mixture, the growth of even very small numbers of pneumococci was not prevented.

Matsunami and Kolmer,[9] using the method of Heist and the Solis-Cohens, incubated meningococci in the whole blood of rabbits, which are immune from meningococcic infection, and of mice, which are fairly susceptible. In rabbit blood little or no growth of meningococci had taken place after twenty-four hours' incubation, but in the blood of the mouse growth was vigorous. Matsunami[10] states in a later communication that he has found evidence that the action of the immune blood on meningococci is truly bactericidal.

Black, Fowler and Pierce,[11] who seeded with undiluted virulent cultures of the typhoid bacillus and of *Bacillus dysenteriae* the blood of rabbits immunized against these organisms and incubated the tubes for different periods of time, found that the organisms were destroyed at the end of five minutes, no bacilli or recognizable fragments being seen in the leucocytes. As the result of comparative studies with various antibodies they conclude that the bactericidal power of blood, thus determined, "is the most dependable criterion of the actual immunity of the animal."

According to Kolmer and Borow,[12] the Heist-Lacy method is a simple method for determining the bactericidal activity of whole blood at intervals after the administration of a drug and yields approximate quantitative results.

### EXPERIMENTS ON MEN

In order to see whether or not bacteria known to be commonly harmful to men would grow more readily in human blood than those which are known to be practically harmless under ordinary conditions, Heist, Solomon Solis-Cohen and Myer Solis-Cohen[13] planted a strain of colon bacilli, isolated from feces, in the whole blood of fifteen healthy men, and a hemolytic streptococcus, isolated from a severe leg ulcer, following a shell wound, in the blood of sixteen healthy men. Almost no growth of colon bacilli had occurred after twenty-four hours' incubation, while vigorous growth of streptococci occurred in all but two of the sixteen human bloods tested. These two experiments they regarded as sufficient to prove that what had been found to be true of animals is also true of man: namely, that there is a certain correlation between the ability of bacteria to grow in the fresh, whole, coagulable blood of the individual or species tested and the virulence of the bacteria for that individual or species.

Myer Solis-Cohen and Heist,[14] in studying individual susceptibility and resistance, worked with many different microorganisms and different individuals. They found great differences in individual responses, indicating either variations in the pathogenicity of various strains of the same microorganism or variations in the personal resistance to a given strain of different human beings. We, therefore, believe it reasonable to infer that the fresh, whole, coagulable blood of any individual will destroy or inhibit the growth of organisms that are nonpathogenic for that individual, while it exhibits no such action upon organisms that are pathogenic for him.

The clinical results of Myer Solis-Cohen[15, 16, 17] and others[18] in cases of inoculation with killed bacteria, the vaccines consisting chiefly of organisms against which the patient's blood showed little or no antibacterial power, also seem to bear out the inference that growth of a given organism in the whole, coagulable blood of an individual indicates susceptibility to that organism, while failure to grow indicates resistance. That bactericidal or bacteriostatic power can be developed by means of inoculation with killed bacteria has been demonstrated experimentally by Solomon Solis-Cohen and Heist,[19] Bull and Bartual,[6] Smiley,[7] Robertson and Sia,[8] Matsunami,[10] and Black, Fowler and Pierce.[11]

### DEFICIENCIES IN THE ORDINARY CULTURE

Bacterial cultures as ordinarily made (with the exception, of course, of those taken from the patient's blood) yield no information as to whether or not the organisms found are really infective. Quite frequently the predominating organism in such a culture is nonpathogenic for the patient, as indicated by the fact that it disappears when grown in his whole blood. Yet most bacteriologists regard the predominating organism as the etiologic factor in an infection and prepare their vaccines accordingly. Consequently, such vaccines at times probably have little or no specific action, whatever may be their value as indifferent proteins.

The ordinary culture may sometimes fail to reveal the etiologic organ-

ism, especially when the latter occurs in such small numbers that it is overgrown or has its growth inhibited by other organisms which are present in larger numbers, but are nonpathogenic for the patient. The latter may, indeed, be of pathogenic varieties; but the point is that, at the moment, they are not pathogenic for the person harboring them, who is a carrier only. Even should a vaccine made in the ordinary way contain among the nonpathogenic varieties a few of the etiologic organisms, the vaccine can have but a minimal specific value, since the dose of the specific antigen bears such a small proportion to the dose of vaccine given as to be practically negligible.

### TEST FOR SPECIFIC PATHOGENICITY

In the pathogen-selective culture, however, the predominating organisms that are nonpathogenic for the patient are destroyed or have their growth inhibited, thus permitting a relatively free growth and multiplication of the organisms that are pathogenic for the patient, even if on the original smears they were proportionately few in number. In other words, even though the whole blood should fail to kill those organisms that are not pathogenic for him, it retards their multiplication, while exaggerating the growth of those organisms that are pathogenic for him. So that on smears from cultures made in whole blood and then recultured on a plain culture medium, the predominating organisms are regarded as pathogenic for the patient, while those found in a small proportion are regarded as relatively harmless for him. This method not only has the advantages of simplicity and promptitude over mixed culture passage through animals in picking out organisms but is also more definitely specific because the organisms are brought in direct contact with the patient's own blood.

The method, of course, has limitations. The reaction is, to a certain extent, quantitative, and is thus affected by the relative proportion of blood and bacteria engaged. When a relatively large number of organisms, nonpathogenic to the patient, are planted in a relatively small quantity of blood, it is conceivable that the amount of bactericidal or bacteriostatic substance present may not be sufficient to destroy or inhibit the growth of all of them; and, if only a few escape, they may multiply rapidly in the absence of any restraining influence. Conversely, if relatively few organisms are planted in a relatively large quantity of blood that is but slightly inimical to them, it is possible that enough of the restraining substance might be present to prevent their growth entirely.

### THE PATHOGEN-SELECTIVE CULTURE

The procedure in obtaining cultures from body recesses, fluids and excretions, is as follows:

For each culture the materials necessary are:

1. One culture tube, containing preferably a rich medium such as Löffler's serum agar.
2. One empty sterile test tube (small).
3. One sterile cotton-tipped applicator.
4. Sterile syringe and needle (gauge 19-23).
5. A Bunsen or alcohol flame.

The primary culture is obtained with sterile applicator and inoculated in a tube of rich culture medium. (This is called the plain culture.) The same applicator is then introduced into the sterile test tube and a very thin film is inoculated on the bottom and side of the tube. Blood from the patient is immediately obtained by venipuncture and about 3 to 5 c.c. are allowed to run into the empty test tube recently inoculated. (This is known as the blood culture.) Where multiple recesses or fluids are to be cultured the procedure is repeated. All are, of course, done simultaneously so that the requisite amount of blood can be obtained by a single venipuncture and distributed to all the empty inoculated tubes at once. Both the plain and blood tubes are then incubated for twenty-four hours, and then the plain cultures are placed in an ice chest to prevent further development. The clotted blood is then removed from the blood tube and, with a sterile platinum loop, the residual blood at the bottom of the tube is inoculated on one or more tubes of plain culture medium in a thin film on the surface and is incubated for twenty-four hours.

At the end of that period the plain cultures previously placed in the ice box and those inoculated from the blood tubes are examined for organisms. Frequently one can at a glance differentiate types of organisms such as streptococci, etc., because of the blood film on the plain medium.

## PREPARATION OF VACCINE

In the preparation of vaccine, subculture should be avoided in order that relative proportions of growth be maintained. The culture tubes are filled to the top of the slants with sterile normal saline solution; the growths are washed off with the aid of a platinum loop and agitation, and the suspension of vaccine is made so that the organisms that grow out on the blood-tube cultures are in a 9:1 proportion to those that are present only on plain cultures.

The vaccine, therefore, will consist chiefly of those organisms that grew out in spite of contact with the patient's blood, with the addition of 10 per cent of organisms that grew up on the plain culture but failed to grow up in the patient's whole blood, so that any possible antigenic effect of symbiosis is preserved.

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## DISCUSSION

*Dr. Herman Spitz.*—I would like to report a little modification of this method that we have been using in gonorrhea cases. We place two or three cubic centimeters of the patient's whole blood on agar slants and then inoculate them with the pus or prostatic secretion. We then remove all growth, wash it, and prepare the vaccine from the washed sediment. We have found that many of the organisms do not grow at all. In giving the vaccine we always advise the physician to use large doses and to get a reaction, not only local but systemic. I feel that unless we get a very good reaction the vaccine is practically useless. The vaccine has been productive of good results by this method in a fair number of chronic infections.

*Dr. O. Lowy.*—I would like to ask Dr. Rubenstone whether he has done any complement fixation in connection with his work. I have been very interested in that, especially in arthritis.

*Dr. Myer Solis-Cohen* (closing).—You must distinguish between the organisms that are infecting the patient and other organisms present. The purpose of the pathogen-selective culture is to inhibit the growth of or to destroy the organisms that are nonpathogenic for that particular patient and to encourage the growth of organisms that are pathogenic for him. This method has been used in arthritis, asthma, endocarditis and in various other conditions, in every condition where there is an infection. The surgeon thinks he has removed the infection when he merely removed the infected tissue. The bacteriologist frequently thinks that he has prepared effective vaccine, when in reality the etiologic organism may never have appeared in his culture, which may have contained only organisms that were nonpathogenic for the particular patient. As regards the administration of vaccine, it may be that in gonorrhea it is all right to give very large doses, but in most other conditions a really specific vaccine must be given very cautiously, according to the reaction from the preceding dose.

# PERMANENT STANDARDS TO BE USED WITH BENEDICT'S "CLINICAL QUANTITATIVE TEST" FOR SUGAR IN URINE*

By Jeanette Allen Behre, Ph.D., and William Muhlberg, M.D.,
Cincinnati, Ohio

ABOUT two years ago Benedict[1] described a "clinical quantitative test" for sugar in urine. At the same time Folin[2] published a "clinical quantitative test" for albumin in urine. These tests have apparently not been published in any scientific journal and so may have escaped the attention of many physicians and laboratory workers who would find them useful. The main object of the new procedures was to provide very simple and relatively accurate methods for the quantitative estimation of small quantities of sugar and of albumin, such as may be responsible for the so-called "borderline" reactions for these substances in urine. Thus the new tests make it possible to record in definite figures the quantities of sugar or of albumin which have heretofore been recorded as "trace," "slight trace," etc., and so to decide upon a more definite basis whether or not the quantities found indicate a pathologic condition. Using these tests it is also possible to follow with accuracy the progress of the patient showing these "borderline" conditions. The sugar test has been used with highly satisfactory results in the laboratories of many of the insurance companies and it would seem to be very practical for use in a wide number of instances where routine examinations are being made, such as in hospitals, private laboratories, etc.

It is the object of the present paper to report the preparation of permanent standard solutions for use in the Benedict "clinical quantitative test" for sugar. Before presenting these findings we feel that it will be of service to present briefly the original directions for the method.

The determination is carried out in test tubes of equal diameter, graduated to 25 c.c. One c.c. of urine, 3 c.c. of 0.2 per cent picric acid and 0.5 c.c. of 50 per cent acetone (prepared fresh each day by dilution of acetone) are added in the order named, and the tube transferred at once to a boiling water-bath and heated for from ten to fifteen minutes, cooled and the contents diluted with water to 25 c.c. As many as ten determinations can be made at one operation. The color is then compared in a side-by-side inspection, by transmitted light, with the color of a set of standards contained in tubes of the same diameter. Benedict recommends that quantities of sugar up to 0.2 per cent, in urines of low specific gravity, or of 0.3 per cent, in urines of high specific gravity (over 1.025), should be considered as normal.

The standards described in the original directions are made by dilution of an alkaline picric and picramic acid solution, and they maintain their color for

*From the Laboratory of the Union Central Life Insurance Co., Cincinnati, Ohio.
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only about a month, which is an inconvenience in routine work. The standards described in the present paper are made of potassium bichromate, chromium potassium sulphate ("chrome alum") and sulphuric acid, and the combination has been found to be perfectly stable when kept in sealed tubes. We have, in this laboratory, tubes which have been kept for a year, much of the time in bright sunlight, and which show no change from the original color.

The color matches that given by glucose solutions, and by urinary sugar, almost perfectly, not only in a side-by-side comparison (by transmitted light), but also when the reading is made in a colorimeter.

The relative amounts of the constituents used vary in the different standards, the formula for each standard having been worked out arbitrarily so that it would most nearly match the color given by a pure glucose solution of the given concentration. For the 0.1 and 0.2 per cent standards it was found that the addition of some cobalt chloride made the reading better in the colorimeter.

The standards are put into tubes of the same diameter as those used in the sugar determination. These tubes are first drawn out at the end, the liquid is introduced through the small opening, and the tubes sealed off so that they are perfectly air-tight. If exposed to the air, the solutions darken.

The directions for making the standards follow:

### DIRECTIONS FOR MAKING STANDARDS

(Chemically pure compounds should be used)

*0.5 per cent Standard.*—8 gm. potassium bichromate dissolved in water with 6 c.c. concentrated sulphuric acid. Cooled. 0.6 gm. chrome alum added and dissolved. Made up to 100 c.c. with distilled water.

*0.4 per cent Standard.*—6.66 gm. potassium bichromate dissolved in water with 5 c.c. concentrated sulphuric acid. Cooled. 0.55 gm. chrome alum added and dissolved. Made up to 100 c.c.

*0.3 per cent Standard.*—4.6 gm. potassium bichromate dissolved in water with 5 c.c. concentrated sulpuhuric acid. Cooled. 0.5 gm. chrome alum added and dissolved. Made up to 100 c.c.

*0.2 per cent Standard.*—0.52 gm. potassium bichromate dissolved in water with 1.5 c.c. concentrated sulphuric acid. Cooled. 0.1 gm. chrome alum and 3.2 gm. cobalt chloride added and dissolved. Made up to 100 c.c.

*0.1 per cent Standard.*—0.075 gm. potassium bichromate, dissolved in water with 1.5 c.c. concentrated sulphuric acid. Cooled. 0.172 gm. chrome alum and 1.48 gm. cobalt chloride added and dissolved. Made up to 100 c.c.

### SUMMARY

Directions are given for the preparation of permanent standards for use in the Benedict "clinical quantitative test" for sugar in urine.

The original directions for Benedict's method are briefly presented.

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# A NOTE UPON A SIMPLE METHOD OF QUANTITATIVE BLOOD CULTURE*

By Robert A. Kilduffe, M.D., Atlantic City, N. J.

THE use of bacteriostatic or bactericidal dye compounds in the treatment of bacteriemias has emphasized the value of determining at times the degree as well as the presence of bacterial blood infections through the enumeration of the bacteria in a known quantity of blood.

This procedure is readily and simply incorporated as a part of routine blood culture by the method in use in these laboratories and described below.

A 10 c.c. syringe is wrapped in paper or muslin, barrel and plunger separated, and sterilized by dry heat. The needle is placed in a stoppered small tube and sterilized separately.

Bacteriologic test tubes are plugged with cotton and sterilized and sufficient 2 per cent sodium citrate in normal saline is added to make a layer about one inch deep in the tube. In similar tubes exactly 2 c.c. of distilled water are placed and both sets are autoclaved.

The specimen is drawn by venipuncture after sterilization of the arm. A known volume, 1 or 2 c.c. or some other convenient quantity, not more than 5 c.c., is ejected into the distilled water tube. The remainder of the blood is ejected into the citrate tube.

On the label of the distilled water tube is noted the amount of blood used.

Both tubes are then brought to the laboratory, and inoculations are made at leisure, though the time interval between collection and inoculation is as short as possible.

Knowing the volume of water and blood, the volume representing one centimeter of blood is easily calculated, and this amount is transferred by a sterile pipette to a sterile Petri dish. Agar, 1.5 per cent, plain or glucose, melted and cooled to 40° C., is then poured, the blood and agar mixed, and the plate allowed to solidify.

From the citrate tube inoculation is made to a flask containing 100 c.c. of 1 per cent glucose broth.

After incubation the plate shows the number of colonies per cubic centimeter of blood, and the broth flask gives sufficient growth for all purposes.

The blood being laked, both depth and surface colonies may be counted.

For slow growing organisms, or where the number is few, growth may be evident in the flask before it is clearly seen on the plate. In such cases a preliminary report is made of the nature of the growth, and the numerical intensity of the infection is reported later.

One culture serves as a check on the other. Information, in positive cultures, is obtained simultaneously both as to the nature and to the intensity of infection.

*From the Laboratories of the Atlantic City Hospital.
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# A MODIFICATION OF THE UNGER BLOOD TRANSFUSION APPARATUS*

By H. W. Jones, M.D., Philadelphia, Pa.

THE Unger blood transfusion apparatus has been used successfully by us in some fifteen hundred transfusions. As originally described, its use requires the presence of two assistants, one to inject the normal saline solution and one to pour ether on the syringe by which the blood is transfused. The following modifications have been made and are herewith described:

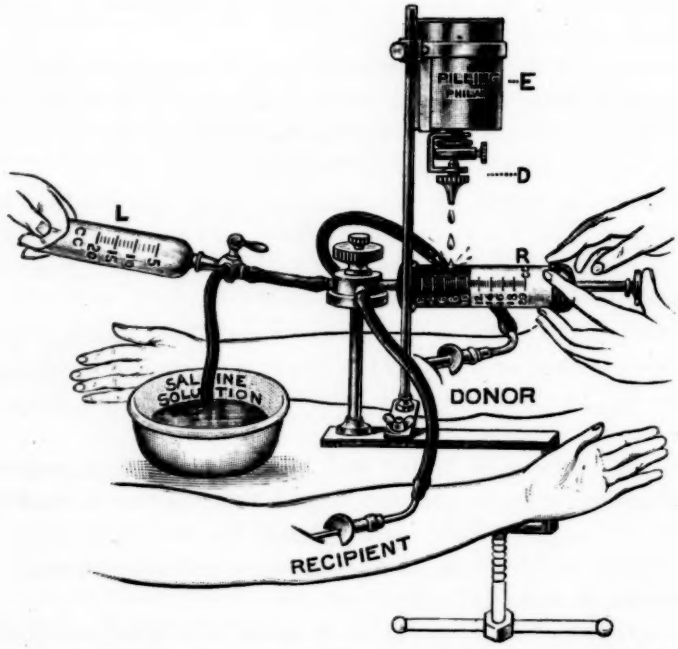


Fig. 1.—*E*, ether; *D*, dropper; *R*, Record syringe for blood; *L*, Luer syringe for salt solution.

1. An attachment, which holds the ether can so that a stream of ether of variable size and force is permitted to fall on the blood-bearing syringe, is shown in the plate. A needle valve, by which the size of the stream can be controlled, is inserted in the top of the can.

2. The caliber of the Unger needle is modified by enlarging the inside diameter. The shoulder is wider, making its position more secure.

3. The curve of the attachment that connects the rubber tubing to the donor's needle is made less. This eliminates a point of resistance with which the blood from the donor's needle comes into contact, thereby lessening the chance of coagulation at that point.

*Received for publication, February 17, 1926.

4. The bed of the screw fastening at the side which holds the inner portion of apparatus in place is made deeper. This prevents movement and displacement, and prevents the entrance of air.

5. A two way stopcock is added to facilitate the filling of the syringe with normal salt solution without disconnecting it.

6. The record syringe is held in place by a bayonet joint. This prevents the syringe from slipping, thereby eliminating another source of air entrance.

7. The bore of the steel connecting tubes to donor's needle and recipient's needle is increased. This eliminates further points with which the blood might meet with resistance in its transmission.

We feel that the ether attachment simplifies the operation because it does away with one assistant and because ether is not wasted unnecessarily. The old method of spraying the ether was most objectionable to the patient.

The other changes lessen the danger of air influx and help to prevent the formation of early products of coagulation.

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## A SIMPLE METHOD FOR FINDING DIBOTHRYOCEPHALUS LATUS EGGS IN THE STOOLS*

By Harry B. Schmidt, M.D., Detroit, Mich.

THERE are a number of complicated methods of examining stools for the eggs of the Dibothrycephalus tapeworm. The method which I have used for the past ten years has been very satisfactory and is time saving, when contrasted to other methods.

The procedure is as follows:

After the stool has been mixed with a small amount of normal saline solution and centrifuged, a small portion from the supernatant and bottom sediment is placed on a slide and a cover-glass is pressed down firmly with a blunt instrument. After some experience one can ascertain just the right amount of pressure to use in order to raise the caps on the eggs. Under the low power of the microscope, eggs with open caps are easily and quickly differentiated from other material in the stool sediment. By this method a slide per minute can be satisfactorily examined under the low power of the microscope.

In nine out of ten cases the stools do not have to be centrifuged, but simply mixed with water and examined as described.

*Received for publication, March 15, 1926.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**Maloney, P. J., and Weld, C. B.: Diphtheria Toxin and Antitoxin Flocculation, (Ramon Test).** Jour. Path. and Bacteriol., Edinburgh, October, 1925, xxviii, 655.

The authors report their investigation of the test devised by Ramon (Ann. de l'Inst. Pasteur, 1925, xxxix, 1), which is based upon the flocculation occurring when suitable mixtures of diphtheria toxin and antitoxin are brought into contact.

The procedure consists of adding to constant amounts of diphtheria toxin (or toxoid), in a series of tubes, varying amounts of diphtheria antitoxin and incubating the tubes at a temperature not to exceed 50° C.

The first tube in which flocculation occurs is termed the "indicating tube," and, according to Ramon, contains a balanced mixture of toxin and antitoxin.

Ramon believes that this test may be used as an in vitro method of antitoxin assay and also as a measure of the relative immunizing power of toxoids.

Inasmuch as conflicting reports have been made by various observers, Maloney and Weld conducted tests upon trial bleedings from 31 horses under immunization. In all, 100 tests were made.

The strength of the antitoxin was estimated by Ramon's method and by the usual animal method for the standardization of antitoxin.

A perceptible divergence occurred in the results, deviations of 200 or more units being noted.

The exact nature of the Ramon test is as yet unexplained.

The authors believe that it has a field of usefulness, the extent of which, however, must be determined by further studies.

In about 80 per cent of antitoxic sera the flocculation unit value is about 20 per cent of the animal value. The test, therefore, may be used as a rough guide to the antitoxin unitage.

The occurrence of flocculation may also serve as an index to the antigenic value of the toxins or toxoids, as those which failed to produce flocculation also failed to produce immunity.

They conclude that the Ramon test cannot be used for the exact determination of the antitoxic value of an immune serum, or for reliable indication or representation of a balanced mixture of toxin and antitoxin.

**Wright, H. D.: The Bacteriology of Acute Infectious Endocarditis.** Jour. Path. and Bacteriol., Edinburgh, October, 1925, xxviii, 541.

The purpose of the investigation was to determine the frequency of streptococcemia in acute infectious endocarditis, in view of the number of cases reported in which sterile blood cultures have been obtained.

The isolation of streptococci from the blood stream was found to be a relatively simple procedure, a wide variety of culture media being suitable. The failure to obtain growth, therefore, and especially when the cultures were sterile on several attempts, might be taken as reliable evidence that organisms were not present in the sample examined and that a bacteriemia did not exist.

Minor variations in technic had no apparent influence on the frequency with which positive cultures occurred.

Either liquid or solid media was suitable. There was no advantage in the use of anaerobic methods.

Media containing trypsin or sodium citrate (not to exceed 0.2 per cent) were slightly superior. The addition of lactic acid was without advantage.

Streptococci may be detected by the usual methods in vogue when present in no greater numbers than 1 to 2 per c.c.

If the specimen is kept at room temperature delay in inoculation is without effect on the viability of the organisms.

Delay in the appearance of growth is due to a prolongation of the latent period and dependent upon peculiarities of the organism rather than upon inhibitory properties of the blood.

Agonal or postmortem cultures were unreliable because of the frequency with which streptococci are concerned in terminal invasions.

The frequency of bacteriemia in endocarditis varies. In some cases there are no bacteria found in the blood stream; in others they are liberated as secondary invaders from the valvular lesions. The blood may also be actively bactericidal. A negative culture, therefore, does not negative the diagnosis.

When the blood culture is positive, bacteria may readily be demonstrated on the valves, in sections. Where cultures are sterile bacteria may also be seen, but they appear to be disintegrated.

The author believes that the streptococcus is the agent of most importance in the causation of acute bacterial endocarditis.

**Brown, J. H.: A Study of Anaerobic Bacteria.** Jour. Bacteriol., November, 1925, x, No. 6, p. 513.

Brown calls attention to the fact that relatively detailed studies of anaerobes may be made with simple test tube methods, and he reports such studies.

He used, for the determination of various biologic characteristics, the simple methods mentioned below.

Cooked meat medium under vaselin was observed for changes in color, consistency, digestion, and gas formation. A black ring at the surface was taken as indicative of hydrogen sulphide formation.

Such cultures were also used for studies of morphology.

Deep agar shake tubes demonstrate the character of deep colonies and give information in a rough way as to the most favorable degree of anaerobiosis. Veal infusion gelatin is incubated at 38° C. and then placed in ice water. Skimmed milk under vaselin and citrated milk are observed for gas formation, coagulation, and peptonization.

Protein digestion is tested by placing 5 mm. cubes of coagulated horse serum, egg white, rennet casein, and fibrin in veal broth tubes which are then autoclaved.

Carbohydrates were sterilized in 10 per cent solution and added aseptically to tubes of sterile broth under vaselin, small bits of rabbit or guinea pig kidney being added to the broth tubes before autoclaving and prior to the addition of the sugar.

The addition of indicators was not found feasible and resort was had to $P_H$ determinations as evidence of fermentation. The best evidence, however, was obtained by adding only 0.2 c.c. of the sugar and, after growth for three months, testing for the presence or absence of sugar by Benedict's test. If fermentation had occurred the sugar was destroyed and there was no reaction.

For mannite, glycerol, and salicin, the culture, after a month's incubation, is heated in a boiling water-bath for fifteen minutes and inoculated with B. coli. The absence of gas formation must be interpreted with caution because of the possible presence of thermostabile gas inhibiting factor; the presence of gas indicates that the anaerobe failed to destroy the small amount of sugar in the medium.

Hydrolysis of milk fat is a useful differentiating point.

Thick cow's cream is washed several times with normal saline and recovered by centrifugation. A thick emulsion is made with normal saline and autoclaved. About 0.5 c.c. is pipetted aseptically into 5 c.c. of broth under vaselin. In time the cream forms a compact

layer 1 to 2 mm. thick below the surface of the vaselin. Hydrolysis is indicated by the transformation of this layer into a thick layer of gray flocculent material composed largely of fatty acids and soaps.

Various chemical titrations may be included and are reported in the study.

The necessity for such studies of the anaerobe group is emphasized and the characteristics of a number of anaerobes so studied are reported. Certain "metabolic groupings" of anaerobes are described.

**Dold, H.: Critical Comparison of the Determination of the Germ Content of Bacterial Vaccines.** Deutsch. med. Wchnschr., November 6, 1925, li, 1851.

Dold discusses the accuracy of various methods which have been proposed for the numerical standardization of bacterial vaccines.

Wright's method he considers inaccurate because: (a) the number of red cells in the blood used for comparison varies; (b) some of the cells and bacteria may be lost in staining; (c) cells may be superimposed on bacteria and these be unnoticed; (d) bacteria may vary in visibility; (e) cellular débris and platelets may be confused with bacteria; and (f) the uncontrolled personal factor.

He believes a more accurate method to be that of Fries in which the bacterial suspension is compared with a standard suspension of yeast by a method similar to Wright's. Some of the criticisms applied to Wright's method apply also to this.

The most accurate method is direct count of the organism in a counting chamber, but this is not applicable to all organisms.

Indirect methods, such as weighing, centrifuging (Hopkins), and nephelometry are all used, but all possess inherent errors.

Inasmuch as the numerical concentration of the organisms does not necessarily indicate the antigenic value of the vaccine; since antigenic substances may be in solution, and since the efficiency of the vaccines depends upon various factors besides their numerical strength, Dold believes that the procedure is impracticable and without scientific basis.

**Finerund, C. W.: Ulcus Acutum Vulvae (Lipschütz).** Arch. f. Dermat. u. Syph., January, 1926, xiii, No. 1, p. 55.

In this case report attention is called to an acute ulceration of the vulva due to an organism originally called B. crassus but since shown to be identical with Döderlein's bacillus.

It is best grown aerobically on serum-sugar-stab cultures or maltose-ascitic-fluid-agar plates on which it appears as delicate hairy outrunners on the borders of abundant and varied growths.

Later it turns grayish-yellow. In older cultures long threads are seen. It is a rather thick, gram-positive bacillus.

The disease is frequently confused with other conditions.

The clinical picture is described in detail, and the paper is illustrated with microphotographs showing the organism and the lesions.

**Schamberg, J. F., Harkins, M. J., and Brown, H.: Gold Compounds in Treatment of Experimental Tuberculosis of Skin in Animals.** Arch. f. Dermat. u. Syph., January, 1926, xiii, No. 1, p. 43.

In an illustrated paper chemotherapeutic studies of a number of gold compounds in skin tuberculosis in rabbits are reported in detail.

The authors believe a favorable, though not curative, effect is produced by gold therapy and that the subject deserves extensive study.

The study was suggested by the work done abroad with "Sanocrysin."

The paper should be read in the original for the details which do not lend themselves readily to abstraction.

**Jaffe, R. H.: Amyloidosis Produced by Protein Injections.** Arch. Path. and Lab. Med., January, 1926, i, No. 1, p. 25.

The production of amyloidosis by repeated injections of nutrose is confirmed in white mice. While amyloidosis may occur in the spleen after 40 injections (daily) of 0.2 c.c. of a 3 per cent solution, 60 injections are required for its uniform production. Larger doses do not hasten its development. After 70 injections even the heart valves may be involved. If the injections are continued, an endothelial absorption takes place in the liver. Human or animal serum may be used with similar results.

Hypochromatic anemia and leucocytosis (polymorphonuclear) occur.

Amyloidosis results from acquired hypersensitiveness to the injected substance.

Two microphotographs and two colored plates accompany the paper.

**Lang, F. J.: Role of Endothelium in the Production of Polyblasts (Mononuclear Wandering Cells) in Inflammation.** Arch. Path. and Lab. Med., January, 1926, i, No. 1, p. 41.

An experimental study of the histogenesis of mononuclear giant cells is reported in detail with 10 illustrations.

The polyblasts arise partly through the mobilization of local histocytes, the resting wandering cells of the loose connective tissue, and partly through the rapid hypertrophy of emigrated lymphocytes and monocytes. The hematogenous cells quickly join with the histocytes in their transformation.

The endothelium does not give rise to ameboid elements but to fibroblasts. There is no production of "endothelial leucocytes."

**LeCount, E. R., and Singer, A. H.: Fat Replacement of the Glycogen in the Liver as a Cause of Death.** Arch. Path. and Lab. Med., January, 1926, i, No. 1, p. 84.

Attention is called to the fat replacement of the glycogen in the liver as the sole autopsy finding and as a cause of sudden death in alcoholics. Two autopsies are reported and eleven cases discussed.

**Corper, H. J.: A Control Method of Staining Smears for Tubercle Bacilli.** Arch. Path. and Lab. Med., January, 1926, i, No. 1, 93.

The necessity for controlling the technic is emphasized and a method suggested for this purpose.

A suspension containing 5 to 10 mg. of bacilli in 1 c.c. of equal parts of normal saline and acetone is prepared by grinding a weighed amount of bacilli in a centrifuge tube with a small glass rod, at first dry, then with saline drop by drop until one-half the total volume of the solution has been added. Then the acetone is added slowly with stirring.

The suspension is kept in a rubber stoppered bottle and shaken before use. It is used to make a control smear with the material to be examined. Due precautions must be taken to avoid accidental contamination of the specimen with the control material.

Both smears are then simultaneously stained in a specially constructed holder so that they are subjected to simultaneous procedures.

**Wilson, W. F.: A Simple Apparatus for Obtaining Cultures of Anaerobes.** Jour. Path. and Bacteriol., Edinburgh, October, 1925, xxviii, 673.

The apparatus presented is a newer form of that originally described by the author in 1917. It is made of porcelain, since the original, being made of tin, was gradually corroded by the action of the caustic soda and pyrogallic acid solution.

It consists of a hollow disc-like container with a central circular opening. At the sides are two nozzles *C* and *D*.

A few teaspoonfuls of dry pyrogallic acid are placed in the cavity of the disc. The inoculated Petri plates, which may be piled one on top of another, are placed on the shelf

made by the edges of the circular opening in the disc. They are then covered by an inverted beaker or jar which is sealed to the disc by plasticine.

*C* is connected to a vacuum pump; *D*, by means of pressure tubing, is connected through a glass T-tube to a gas tap and to a glass tube inserted into a jar containing the alkaline fluid described by Rockwell: acid sodium phosphate, 0.75 gm.; sodium bicarbonate, 50 gm.; water 500 c.c.

The tube at *D* is clamped and air drawn out of the apparatus. The clamp attached to the gas tap is then released and gas passed through the apparatus so that it fills and empties twice. A vacuum is then created and 20 c.c. of the alkaline fluid drawn into the main cavity of the disc, when the tubes leading from *C* and *D* are clamped off and the apparatus incubated.

**Menninger, W. C., and Holder, E. H.: The Relation of Total and Polymorphonuclear Leucocyte Counts in Chronic Appendicitis.** Ann. Surg., December, 1925, lxxxii, No. 6, p. 960.

The authors report a series of 93 cases of chronic appendicitis in which the diagnosis was corroborated by microscopic studies of sections.

The leucocytes were charted by the method devised by Gibson, which applies the observations made by Sondern in 1905 that the total white cell count may be taken as an index of the degree of bodily resistance and the polymorphonuclear count as indicative of the severity of the infection.

Taking 10,000 as the outside normal limit of the total white cell count, and 75 as the normal extreme of polymorphonuclear percentage, the total count is charted on the right side of the graph and the percentage count on the left. For each 1,000 increase in the total count there should be an increase of one per cent in the polymorphonuclears. When the increase is thus proportional a line drawn from one side of the chart to the other will approach the horizontal. A rising line indicates a proportionally poor resistance and vice versa.

The authors use the term "resistance index" to express the disproportion between these two factors. The results of their studies: a falling line in chronic appendicitis as opposed to a rising line—a poor resistance index—in acute appendicitis and general peritonitis.

**Mock, H. E., and Ellis, J. D.: Trauma and Malignancy.** Jour. Am. Med. Assn., January 23, 1925, lxxxvi, 257.

The possible relation of trauma to the production of neoplasms has become a matter of importance in connection with compensation cases. The authors endeavor to epitomize the available data concerning this question and suggest, as a result, that before it can be said positively that a malignant tumor is the direct result of a single trauma, the following postulates should be fulfilled:

1. Definite description by the reporting surgeon of the trauma at the time it was sustained.

2. Definite proof, by every possible means of examination made at the time the injury was sustained, that no tumor already existed at the site of trauma.

3. Definite signs and symptoms of a pathologic process continuing at the site of the trauma until a malignant tumor appeared and was positively diagnosed.

Thus far, they have been unable to find a single case fulfilling these requirements.

To constitute reasonable proof of trauma sufficient to cause definite tissue changes they suggest:

1. Reasonable proof of a trauma of sufficient seriousness to cause definite tissue changes;

(a) The injury to be reported within a reasonable period after the accident.

(b) The examination and condition of the traumatized tissue to be reported by a physician within a reasonable period after the injury.

2. The developing neoplasm must be at the same site as the original injury and must involve some of the tissue which, without reasonable doubt, could have been involved in the original trauma.

3. Definite evidence must be produced to prove that no neoplasm existed at the site of injury prior to the accident.

4. In addition to the history of trauma there must be a history of definite bridging signs, such as a persistent swelling, an unhealed wound, or anatomic and functional disturbances which connect the trauma with the malignant growth; subject symptoms, such as complaint of pain, tenderness, or weakness, are impossible to evaluate or visualize and therefore must not be considered competent evidence.

5. The time that has elapsed between a given trauma and the development of the malignant tumor need not be considered if the foregoing conditions have been present; however, it is safe to say that a malignant tumor that develops within two weeks after the trauma existed prior to the injury.

6. A preexistant malignant tumor may be aggravated or accelerated in its growth by a trauma; the trauma may be the first factor to call the patient's attention to the tumor.

7. In the case of metastatic or secondary tumor developing at the site of trauma, the first four postulates must be met before the employer is held responsible, and such responsibility should be limited to the treatment of the local condition; it should not be extended to include responsibility for the death that is bound to occur shortly, as the trauma could not have aggravated the primary growth.

Illustrative cases are cited in which the relation between trauma and neoplasm was thus reasonably indicated and which were considered compensable.

**Grott, J. W.: The Titration of Gerhardt's Reaction in the Urine in Comatose Conditions.** Presse méd., Paris, November 14, 1925, xxxiii, 1507.

Quantitative determination of acetone in the urine is a tedious and complicated procedure. The author, therefore, makes use of Gerhardt's reaction for aceto-acetic acid, to which, by the procedure described below, he has endeavored to impart a quantitative factor. This test becomes positive, according to Grott, when the excretion of acetone and aceto-acetic acid reaches four grams daily.

To 10 c.c. of urine add 10 drops of iron perchloride solution and titrate with N/10 sulphuric acid until the color is cherry red. A 1:2000 solution of antipyrin is used as a color standard.

The amount of acid required is calculated for 100 c.c. of urine and is termed the concentration factor.

The reaction is based upon the transformation of aceto-acetic acid into acetone by the action of sulphuric acid.

Salicylates or pyramidon must not be administered prior to the test.

The test may also be made by placing 10 c.c. of antipyrin solution in a 10 c.c. graduated cylinder and adding 20 drops of iron perchloride.

In a similar cylinder place 5 c.c. of urine and 10 drops of iron perchloride and add the N/10 sulphuric acid until the color of the two solutions matches. To calculate the concentration, the amount of sulphuric acid required is multiplied by 20.

In a study of 87 cases of advanced diabetes it was calculated that when the concentration rises above 120 there is a possibility of coma.

**Belding, D. L.: The Influence of the Antigen upon the Wassermann Reaction in Pregnant Women.** Amer. Jour. Syph., October, 1925, lx, 4.

Report of a clinical and serologic study of the comparative results with plain alcoholic, acetone insoluble, and cholesterolized antigens in the Wassermann test in pregnant women.

Under two methods of fixation comparable results were obtained when the same cholesterolized antigen was used, but considerable variation occurred when different antigens were employed. Approximately one-third as many positives occurred with the plain antigen, and two-thirds as many with the acetone-insoluble antigen as with the cholesterolized antigen.

A strong reaction with plain alcoholic or acetone-insoluble antigens employed in the technics described almost invariably denotes syphilis; a weak reaction is undecisive.

Strong reactions with cholesterolized antigens do not indicate syphilitic specificity but are less likely than weak reactions to be nonsyphilitic. Cholesterolized antigens should be controlled in pregnancy by plain or acetone-insoluble antigens.

The Wassermann tests were all made by both the Massachusetts Board of Health method and the method devised by the author.

**Török, G.: Reduction Index of the Urine of Atrophic Infants.** Monatschr. f. Kinderh., November, 1925, xxxi, 169.

The observation that the relation between the superficies and body weight is disturbed was the starting point of these investigations, the purpose of which was to endeavor to find some characteristic factor which, in the hands of clinicians, might prove a guide to therapy.

By the reduction index is understood the amount of N/10 potassium permanganate solution reduced by 1 c.c. of urine in 10 minutes' boiling.

The author prefers an indirect method by steam.

In atrophic infants showing a striking gain in weight after receiving carbohydrates, a falling index (lessened amount of organic substances) indicates the start of continuing tissue formation.

Tissue destruction causes a rise in the reduction index.

The author considers the reduction index a delicate gauge of improvement in these cases.

**Elwyn, H.: A New Explanation for the Occurrence of Eclampsia in Pregnancy.** Amer. Jour. Obst. and Gynec., November, 1925, x, 5.

Elwyn suggests that the clinical phenomena of eclampsia in pregnancy and the histologic changes seen in the liver and kidneys in many ways form a complex which parallels that of acute diffuse glomerulonephritis with acute convulsive uremia, though the two conditions are essentially different.

In pregnancy it has been shown that there is an increased irritability of the neuromuscular mechanism of the uterus. For the function of vasoconstriction there is a somewhat similar anatomic mechanism, the impulses traveling along the same neural pathway as those for uterine contraction.

Elwyn advances, as an explanation of the etiology of eclampsia, a hyperactivity of both these mechanisms in response to various stimuli, thus leading to a general arterial constriction which produces the symptom-complex recognized as eclampsia.

**McClure, C. W., Mendenhall, W. L., and Huntsinger, M. E.: Studies in Liver Function, IV. A Procedure for the Uniform Stimulation of the Biliary Flow.** Boston Med. and Surg. Jour., December 3, 1925, cxciii, No. 23, p. 1052.

Magnesium sulphate solution, which is mainly depended upon to produce a flow of bile, at times fails to do so, because, when it is improperly used, it causes a depression of the biliary function of the liver.

In a search for a substance which would uniformly stimulate the flow of bile the following procedure was devised:

After the passage of the duodenal tube into the second part of the duodenum, which is verified by the fluoroscope, the subject reclines on the right side, and a mixture of 5 c.c. of oleic acid and 45 c.c. of water is poured through the tube into the duodenum. The proximal end of the tube is closed by a clip for fifteen minutes. Collection of duodenal contents is then begun by siphonage.

The first sample contains oleic acid and frequently gushes out of the tube. It is discarded. Collection for analytical purposes is then begun and continued for thirty minutes. If, at any time during this period, a marked change occurs in the color, a new flask is sub-

stituted and the differently colored contents are collected for a further period of thirty minutes.

Studies were made of 12 normal subjects of both sexes, ranging in age from twenty-three to fifty, and the results of the analyses are discussed in detail.

**McClure, C. W., Huntsinger, M. E., and Montague, O. C.: Studies in Liver Function, III. Methods for the Determination of the Furfurol Number and the Bilirubin Concentration of Duodenal Contents.** Boston Med. and Surg. Jour., December 3, 1925, excii, No. 23, p. 1050.

*Determination of the furfurol number.*—Reagents: Furfurol (Merck's reagent), sulphuric acid, C. P., Schuchardt's preparation of glycocholic acid.

1. Three-tenths per cent furfurol solution. Dissolve 3 c.c. of furfurol in about 700 c.c. of water in a volumetric flask and dilute to 1 liter.

2. Fifty per cent sulphuric acid. Equal parts of sulphuric acid (Sp. Gr. 1.84) and water. Cool before using.

3. Glycocholic acid standard. Dissolve 100 mg. in about 30 c.c. of N/10 NaOH in a 50 c.c. volumetric flask and dilute with the same solution to 50 c.c.

Method: To 1 c.c. of duodenal contents add 9 c.c. of 95 per cent alcohol and mix. Filter through dry paper. Add 1 c.c. of filtrate to 6 c.c. of 50 per cent sulphuric acid in a test tube. Add 1 c.c. of 0.3 per cent furfurol and 1 c.c. of water and mix by rotation. Place the tube in a water-bath at 67° C. for thirty minutes. Remove and cool under tap water.

If necessary, clarify by centrifugation. A slight turbidity does not interfere.

Prepare a standard by adding 1 c.c. of standard glycocholic acid solution to a mixture of 6 c.c. of 50 per cent sulphuric acid, 1 c.c. of 0.3 per cent furfurol, and 1 c.c. of 95 per cent alcohol. This tube is placed in the water-bath and treated like the unknown.

Color comparison is made in a Hellige colorimeter, using the scale reading up from the bottom.

The reading of the unknown multiplied by 2 is arbitrarily termed the furfurol number.

*Determination of Bilirubin.*—By bilirubin is meant the brownish-colored pigment or mixture of pigments seen in the duodenal contents after stimulation by magnesium sulphate or oleic acid.

Reagents: Ten per cent suspension of calcium hydrate in water. One-half per cent solution of sulphanilic acid (5 grams of sulphanilic acid dissolved in about 800 c.c. of distilled water and diluted to 1 liter in a volumetric flask. Add a few c.c. of chloroform to prevent growth of fungi. The solution is stable.) Two-hundredths per cent solution of sodium nitrite (0.1 gm. in 500 c.c. of water). Two-tenths per cent solution of biliphein (10 gm. in 50 c.c. of chloroform. The solution is stable for at least 2 months). HCl solution: 25 c.c. of C. P. HCl and 75 c.c. of distilled water. Magnesium sulphate solution: 25 grams in 100 c.c. of water.

Method: Place 10 c.c. of duodenal contents, if yellow (5 c.c. if brown) in a 15 c.c. centrifuge tube. Add 1 c.c. of magnesium sulphate solution and 3 c.c. of calcium sulphate suspension and stir with a glass rod. Rinse the rod with water and centrifuge. Decant the clear fluid and add 5 c.c. of water to the precipitate; mix with glass rod, and add 5 c.c. of HCl solution. Mix until all the calcium hydrate is dissolved. Wash the rod with water and centrifuge. The supernatant fluid should be opalescent and should not give any color with the sulphanilic acid-nitrite reagent.

Again decant; add 10 c.c. of alcohol; mix, grinding up the flocculent particles; centrifuge again till fluid is clear. Repeat the alcohol washing three times. After the third washing decant the alcohol; add 3 c.c. of chloroform, and mix with a glass rod. Filter through paper. Add 1 c.c. of this chloroform filtrate to the following mixture, prepared exactly in the order described: 1 c.c. of 0.5 per cent sulphanilic acid solution, 1 c.c. of 0.02 per cent sodium nitrite solution, and 4 c.c. of alcohol. Mix by rotation and allow to stand thirty minutes.

Compare against the color developed in 1 c.c. of standard biliphein solution similarly treated.

The index of bilirubin concentration is expressed in terms of mg. per 100 c.c. of duodenal contents.

Biliphein is a purified bilirubin prepared by Eimer and Amend.

**Tandowsky, R. M.: Serologic Studies of Proteinurias: Results of Precipitin Tests Following Injections of Antistreptococcus Serum and of Normal Horse Serum.** Jour. Am. Med. Assn., Jan. 23, 1926, lxxxvi, 263.

Proteinuria is neither inevitably significant of nor concomitant with renal disease. It may follow the intravenous administration of large amounts of serum.

The paper reports the results of precipitin tests on the urine of patients receiving antistreptococcus serum and from dogs after the intravenous administration of normal horse serum.

Rabbits were immunized against the serum and their serum used when the titer was not less than 1:60,000.

To 3 c.c. of urine 0.2 c.c. of rabbit serum was added and readings made after one hour at room temperature.

By this procedure the elimination of horse serum was detected in the urine within thirty minutes. The greatest concentration was found within the first eighty minutes.

The proteinuria continued for approximately four hours.

**Prucha, M. J., and Brannon, J. M.: Viability of B. Typhosum in Ice Cream.** Jour. Bacteriol., January, 1926, xi, No. 1, p. 45.

Reports of a bacterial count of ice cream containing B. typhosum artificially introduced.

After five days a reduction of 90 per cent in the number of typhoid organisms was noted; after twenty days the reduction was more than 95 per cent. After one year one germ in a thousand survived; after two years one germ in ten thousand.

Viable typhoid bacilli were found after two years and four months, when the examinations were terminated because the sample had been exhausted.

Storage was at an average temperature of minus 4° F., and the experiment demonstrates that the typhoid organism is viable after prolonged exposure to this temperature.

**Roffo, A. H.: Serologic Test for Cancer.** Prensa med. Argentina, October 10, 1925, xii, 481.

To 2 c.c. of clear serum add 5 drops of 0.5 per cent solution of neutral red. Normal serum remains yellowish; cancerous serum rapidly turns red.

The reaction is based upon the relation assumed to exist between the colloidal content of the serum and the occurrence or disappearance of fluorescence.

The test was devised in conjunction with an experimental study of tumors in rats.

**Crowell, M. J.: Morphological and Physiological Variations in the Descendants of a Single Diphtheria Bacillus.** Jour. Bacteriol., January, 1926, xi, No. 1, p. 65.

From the study of single cell cultures of B. diphtheriae the following conclusions are formulated:

1. Morphologic types of the diphtheria bacillus have no hereditary significance and no relation to virulence.
2. The descendents of toxic organisms may be toxic or nontoxic.
3. The nontoxic strains are direct mutations from toxic strains.
4. The nontoxic strains are permanently nontoxic.

# BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

## *Applied Biochemistry**

BIOCHEMISTRY has rapidly advanced from a pure to an applied science. In its earlier stages the authoritative works, such as Hammerstein's were written along the line of abstract chemical studies. Perhaps the first outstanding attempt to correlate the purely scientific with the clinical was that made by Mathews. The next great step in this correlation was typified in MacLeod's "Physiology and Biochemistry in Modern Medicine."

The work of Morse carries the development a step farther. He emphasizes perhaps more pure biochemistry and less the clinical aspects than have some other writers. Illustrations and illustrative diagrams are numerous and remarkably well done. A distinct innovation is the inclusion of photographs of leading biochemists which are scattered through the book in the sections in which they have done outstanding work. In this way the student can come to think of the men of whose work he reads in a less abstract way. Probably it would be impossible to accord this honor to all who deserve it, but we regret not seeing a photograph of Dr. Folin, whose name is perhaps better known throughout the profession at large than any of the others.

In his introduction the author has an interesting "family tree," tracing the origin of American biochemistry through some of the lines of its development from Lavoisier in France and Liebig and Voit in Germany, through their pupils such as Atwater, Lusk, Folin, Mandel, Chittenden, Jones and Mathews, and on down through the students of these latter, who have contributed outstanding advances to our knowledge of biochemistry. While made up as an interesting genealogic table it constitutes one of our best briefs for the good effect of *environment,* expressed in this case as a thorough "grounding" under the masters.

The body of the book is arranged in the customary subdivisions: enzymes and their action, the body and its maintenance, glucids, lipids, protids, the special chemistry of the tissues, chemistry of common foods, digestion and absorption, intermediate metabolism, nutrition, the energetics of nutrition, metabolic adjuvants, the excretions, methods for the determination of blood constituents, and metabolic studies on blood and urine.

Scattered through the work are found experimental exercises for the student, illustrative of points brought out in the text. In the appendix the author has incorporated a comprehensive list of reagents and various tables, such as the hydrogen-ion concentration of various substances, characteristic biochemical configurations, scheme for the analysis of milk, table of magnifications, tables for metabolic determinations and vitamin tables.

*Applied Biochemistry. By Withrow Morse, Ph.D., Professor of Physiological Chemistry and Toxicology, Jefferson Medical College, Philadelphia. Cloth. Octavo. 257 illustrations. Pp. 958. Price $7.00 net. W. B. Saunders Company, Philadelphia and London. 1925.

## *Handbook of Pathology**

THIS book was written, in the words of the author, "with the object of presenting in a short, concise form the more important and salient points in pathology," and it is intended primarily for the medical student.

The task outlined has been very successfully accomplished.

The arrangement of the book is, as far as possible, along etiologic lines and, where this is impossible, on the basis of the character of tissue change whereby lesions of similar or allied nature are grouped together.

Beginning with the morphology of cells, degeneration and allied changes (edema, hyperemia, atrophy, hypertrophy, hemorrhage, pigmentation), the reaction to inflammation, etc., are next considered.

Diseases due to bacteria, filterable viruses, metabolic disturbances and those affecting the blood are next discussed, the final chapter of 194 pages being devoted to a consideration of tumors.

Only the more common conditions are described, special attention being given to the morbid anatomy.

The general plan includes a description of the etiology and gross and microscopic features together with such particular features as are of importance in the individual case.

The volume is very well and clearly written. The illustrations, mainly microphotographs, are well chosen and well reproduced.

While not supplanting the more complete and standard texts on this subject, this book furnishes a very satisfactory and useful concise review and reference work of use both to the student and to the physician. It can well be recommended for this purpose.

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## *Parasitology for Medical Students†*

PARASITOLOGY is a subject the mastery of which requires more extensive and profound study than it receives from the average practitioner. It is essential, nevertheless, that some knowledge be had of the salient features of those parasites which are of practical importance from the standpoint of the physician, and in this small volume the author has endeavored, with marked success, to present a brief but practical account of such parasites, their life history, salient characteristics, and a brief discussion of the diagnosis, prophylaxis, and treatment of the diseases caused by them.

The resultant compact volume is very practical.

The descriptions are clear and well written. The illustrations, all micrographs taken by the author, are well reproduced and well chosen.

No one will become a parasitologist through the perusal of this book, but every practicing physician can read it with profit.

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*Handbook of Pathology. By C. Y. Wang, Professor of Pathology, University of Hong Kong. Cloth. Octavo. 282 illustrations, 6 in colors. 513 pages. Price $7.00. William Wood and Co., New York.

†Parasitology for Medical Students. By A. M. Kennedy, Professor of Medicine, University of Wales. Cloth. 61 microphotographs. 1 colored plate. 138 pages. Price $3.00. Oxford University Press.

The subjects covered are: Insects and Arachnoids, Tapeworms or Cestodes. Flukes, or Trematodes, Roundworms or Nematodes, Protozoa, and Spirochetae and Fungi.

Under each heading only those parasites of predominant importance as etiologic agents of diseases relatively common in man are considered and the form followed is the same for all: description, diagnosis, prophylaxis and treatment.

The treatment is briefly given, generally only one method being described. For the treatment of syphilis the reader is wisely referred to larger works.

This is a book which can be added to the library of any practitioner with little doubt of its applicability to his work. For the medical student, for whom it is especially written, it is one of the best small manuals which the reviewer has seen.

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## *Insects and Diseases of Man**

IN this book the author presents in a concise and practical way a discussion of medical entomology for the student, the physician, and the health officer.

No attempt is made to cover the subject in extenso. The aim, rather has been to consider the subject from the standpoint of the essentials for public health work. The task attempted has been very satisfactorily achieved.

The book is divided into two parts: Part I (227 pages) is devoted to the classification, identification, anatomy, life history, etc., of insects of importance in medical entomology, and it includes a section on Arachnidae and one on rodents, together with a short chapter on technic.

There are numerous clear-cut line drawings, many of which were made by the artist of the hygienic laboratory, under the author's personal supervision.

Part II (98 pages) discusses the diseases in the human being which are transmitted by arthropods. It contains a practical discussion of the subject, especially with regard to prevention and control, treatment of carriers, etc., and instructions concerning the collection of material and the details of the necessary field investigations. The book is eminently practical and should be of great use to the student, the health officer, and others interested in or engaged in this work.

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## *Micromethods†*

THIS is a collection of the methods used by Pincusson in the city hospital in Berlin. Some of them are his own and some are his modification of other methods. Most of the determinations are made by means of the colorimeter or the nephelometer, and most of the organic and the inorganic constituents of the urine and blood are covered. Hijmanns van den Bergh's test for bile pigments and the indicator method for hydrogen-ion concentration are

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*Insects and Diseases of Man. By Carroll Fox, Associate Professor Hygiene, Medical School, University of the Philippines. Cloth. 92 illustrations. 249 pages. Price $4.00. P. Blakiston's Son and Co., Philadelphia.

†Micromethods; quantitative determination of the constituents of urine and of blood in small quantities. By Ludwig Pincusson. Geo. Thieme, Leipzig, 1925.

included. Very few of the tests are credited to American authors, though Folin's method for amino nitrogen and for blood sugar, Folin and Wu's method for uric acid, Benedict's method for uric acid, and van Slyke's method for alkali reserve of the blood are found. The author describes and gives a picture of a modified torsion balance by which a weighing accurate to one milligram can be made in a few seconds. This is especially useful in some of Bang's methods in which a piece of moistened filter paper must be weighed quickly before evaporation takes place.

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## *A Textbook of Medical Diagnosis**

THIS book, intended as a practical aid to the practitioner in the study of the diagnostic problems with which he is daily confronted, is an extensively revised third edition of a work originally published in 1911 by two well-known teachers, and it embodies the practical application of their combined experience.

Recognizing that the diagnosis of disease is evolved from the recognition and evaluation of distinctive symptoms for the detection of which many varied and specialized forms of examination may be utilized, the authors propound very clearly a systematic plan of investigation for the clinical study of disease, in which the salient features of each condition are first separately considered and then are grouped under subheadings: "Laboratory Diagnosis," "Summary of Diagnosis," and "Differential Diagnosis," the latter frequently in the form of or accompanied by succinct and useful tables.

The text amply reflects the professional experience of the authors.

The subjects covered include: Respiratory diseases (142 pages); circulatory diseases (257 pages); diseases of the digestive system (246 pages); the urinary system (82 pages); acute infections (239 pages); animal parasites (47 pages); constitutional diseases (20 pages); endocrine diseases (104 pages); and nervous diseases (200 pages).

The discussions are clear, well written, and eminently practical. The section concerned with the clinical utilization and application of blood chemistry might, perhaps, be expanded with benefit.

The volume is concerned entirely with clinical methods of examination; laboratory methods are referred to, and their clinical applicability to the problem at hand and their interpretation are discussed, but the technic is wisely left to volumes particularly concerned with these methods. The Wassermann technic, nevertheless, is described in full, and, as this is a matter beyond the province of the practitioner, it seems superfluous. Precipitation reactions in the serodiagnosis of syphilis are both wisely and conservatively discussed and will be in entire accord with the statements made by the majority of serologists. The section on constitutional diseases is somewhat compact.

The book is easy to read and well printed. The illustrations are numerous, well chosen, illustrative, and well reproduced.

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*A Textbook of Medical Diagnosis. By J. M. Anders and L. N. Boston. Ed. 3. 555 illustrations, 21 in colors. Pp. 1366. Price $12.00. W. B. Saunders & Co., Philadelphia.

There are almost no typographical errors, though one notes *precipitan* for precipitin on page 383 and *Hellar* for Heller on page 521.

The volume should be a useful addition to the reference library of the practitioner, to whom it is addressed.

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## *Handbook of Biological Methods**

ABDERHALDEN'S handbook is one of those immense compilations which can be published only in Germany. Every library should have it, and every one doing biologic work of any kind should have access to it, as it contains everything he may want to know about methods of work.

Lieferung 142 contains 260 pages, is written by Francis G. Benedict, the well-known investigator of metabolism, and contains a description of the respiration calorimeter in the nutrition laboratory of the Carnegie Institution in Boston. Full and detailed descriptions of the methods of determining the amount of oxygen consumed, the carbon dioxide excreted, and the heat given off are given, together with mathematical formulas and tables to simplify the necessary calculations. The fact that it is written by Benedict is sufficient assurance of its high quality and accuracy.

Lieferung 158 contains special methods of colorimetry ranging from a colorimeter for work with large animals, by J. W. Capstick of Cambridge, England, to a microcalorimeter for determining the heat produced by cells, such as red blood corpuscles, eggs of the sea urchin, bacteria, yeast, etc., by Otto Meyerhof of Berlin. There is also an article on the method of determining the gas exchange in large animals, by W. Klein and Marie Steuber of Berlin, and a description of an electrical compensating calorimeter by Paul Hari of Budapest.

Lieferung 143 contains an article by R. Grammel of Stuttgart on the theoretic basis of the mechanics of joints. It is a mathematical treatment of the mechanics and the dynamics of the movements of joints and of the masses moved by them. There is also a short article by E. Hirt of Münich on a graphic method for demonstrating the cause of normal and pathologic voluntary movements, from a simple finger movement to the complicated and coordinated movements of writing.

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*Handbook of Biological Methods. By Emil Abderhalden. Division IV, Part 10, Part 3, (Lieferung 142); Part 4, (Lieferung 158); and Division IV, Part 10, Part 3, (Lieferung 142), Urban and Schwarzenberg, Berlin, 1925.

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## EDITORIALS

### *Laennec*

1781-1826

LAENNEC, like Osler, was of Celtic origin. A great deal in common can be noted in the lives of these two men. Both, as boys, delighted in nature studies and in learning. The Gaelic and the humanities fascinated them. As physicians, both men delighted in pathologic anatomy in the search for Truth and to each the study of tuberculosis afforded absorbing interest. Both of these famous physicians made exact observations and were averse to speculation. To each, medical history was of the greatest importance, and with it they illuminated their excellent lectures and treatises. While Laennec immortalized himself by his anatomic studies and clinical writings and the invention of the stethoscope, it was Osler who first suggested the binaural instrument. Laennec resembled another Celtic physician, Oliver Goldsmith. Both of poetical temperament, they had the same passion for natural history and for playing the flute.

Laennec, as a boy, was fortunately brought up by an uncle who was a physician in Nantes. It was this uncle who, regretting his own scant knowledge of Greek, encouraged the boy to master this language, so that later in life he could read Hippocrates in the original.

Just as the father and mother of Pasteur had worried over their son's passion for pastel drawing, so we find the uncle of Laennec anxious over the boy's delight in, and time spent upon, natural history and poetry. It was no doubt the attention to detail which Laennec thus cultivated that enabled him to discover the subdeltoid bursa, the fibrous capsule of the liver, peritonitis, melanotic cancer, bronchiectasis, the unity of tuberculosis, and many other conditions which he was the first to describe.

Laennec himself suffered many diseases, and one of these may have been pulmonary tuberculosis. His life was a continued struggle against asthma. He employed bizarre remedies, such as sitting at his studies with his body naked. We recall that Benjamin Franklin, who had also suffered from a pulmonary complaint, had advocated and practiced a similar measure. Laennec wrote an accurate description of an attack of angina pectoris from which he suffered. Gout, dizziness, and neurasthenia, he also described, as a victim, and to remedy these "he wearied his legs to rest his head."

Laennec was not so austere as he wished to appear. Called upon for an impromptu toast at a dinner at Soissons, he stated that he was only acquainted with songs pertaining to drinking. He "toasted" tobacco and the inventor of the bottle, quoting Hippocrates that water was the cause of all evil!

Laennec was fortunate in his early medical-student days, in Paris, to come under Corvisart, who was then teaching clinical observation and the method of percussion of Auenbrugger. The famous physiologist, Bichat, was another teacher; and Bayle attracted Laennec by his love of natural history and of religion, and through his careful anatomic study of the tubercle. It was Laennec's faithful devotion to autopsy study that enabled him to become the foremost physician of his day. Pasteur has taught us to reverence great men, and it is in this spirit that the centenary of Laennec's death is now being observed by many medical societies. Laennec, unlike Pasteur, did not live to reap the reward of his genius.

The immortal work on auscultation, which was published in 1819, was met with ridicule by Broussais, and physicians who employed the stethoscope were called charlatans. We, however, as physicians, can well attest the victory of Laennec, when we recall a paragraph from the introduction to his treatise: "I shall consider it ample, yea, more than sufficient reward for my labor, if it should prove the means by which a single human being is snatched from untimely death." —*G. B. W.*

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## *Laboratory Diagnosis*

PROGRESS in medical science is more often the end-result of perspiration than of inspiration, of evolution than of revelation.

The practice of medicine revolves around and evolves from the study of disease and its manifestations; it begins with diagnosis and progresses with

an understanding of the underlying pathology and mechanism of symptom production.

To this detailed study of disease have been applied all the resources of a varied array of sciences, and through their application has arisen a variety of procedures, all of which, to be of use, must be interpreted and clinically evaluated in terms of the individual problem.

Chemical, bacteriologic, and serologic studies have been of inestimable value in the elucidation of many heretofore obscure factors of etiologic and pathologic importance, and from their careful analysis certain general dicta of practical value in diagnosis have been formulated. There is an unfortunate tendency, however, which cannot be too often commented upon nor too strongly decried, to regard the test as the paramount thing, to overlook the vital necessity for its interpretation.

There are too many and too glib references in current medical conversation and current medical literature to "laboratory diagnosis" and "diagnostic tests."

On the one hand, there are those who tend to rely almost entirely upon the results of laboratory investigations, and, on the other, those who remind us that laboratory aids often do more than furnish a verification of that which the well trained bedside clinician can discover through the peculiar technic or devices characteristically his own.

Neither is entirely right, and it is unfortunate that the bulk of the profession is to be found ranged either on one side or the other rather than in the middle of the road.

*There are no pathognomonic laboratory tests! The purpose of the laboratory is not to make diagnostic ventures!*

No one is more insistent than the laboratory worker in proclaiming from the housetops the essential necessity for a correlation of laboratory and clinical data and their joint interpretation. The laboratory investigations are only *one* phase in the study of the individual case, one means of securing information frequently obtainable in no other way, one more item among the many whose sum total is the diagnosis.

It is by the well trained bedside clinician that the results of laboratory studies are most often intelligently interpreted and laboratory studies most skillfully applied, but even the well trained bedside clinician sometimes fails to detect hidden foci of spirochetae whose presence is revealed by the complement-fixation reaction; may fail to differentiate the early chancre from the chancroid in the absence of the dark-field illuminator; may overlook the presence of immune bodies detected by the Schick or Dick tests; may experience some difficulty in the immediate differentiation of uremic from other comatose states without the assistance afforded by blood chemical determinations, and so on.

It is when these and other similar situations arise that the peculiar technic or devices characteristically the clinician's may be inadequate to diagnosticate the condition clearly and completely, and then the laboratory is of the greatest value and assistance.

Unfortunately, there exists a modicum of those not so skillful or so well trained, and it is by these that a blind reliance is too frequently placed upon the laboratory and its tests.

The laboratory requires no clinical accolade. Properly used and skillfully applied to the scientific study of clinical problems, it furnishes both valuable and useful information and is an indispensable part of the study of those problems.

When improperly applied and its results abused or unwisely interpreted, the onus rests upon the clinician and not upon the laboratory.

—*R. A. K.*

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## *CORRESPONDENCE*

To the Editor

Dear Sir:

Every clinical pathologist certainly must agree with Dr. Haden's editorial statement in the April number of your Journal: "That there are wide differences in normal hemoglobin values as given by authors and workers." This has proved so difficult in correlating hemoglobin estimations on the same patient, when made in different places, that in this hospital over two years ago, we began reporting all hemoglobin estimations in grams per 100 c.c. For those who are wedded to a given standard, it is easy to translate the approximate percentage by a simple multiplication, but many soon acquire the ability to visualize the hemoglobin value in grams, rather than percentage, just as the cell counts, which are given in actual values, have long since acquired definite concepts in the physician's mind.

I would suggest, therefore, that instead of trying to arrange an authoritative hemoglobin percentage standard, the American Association of Clinical Pathologists and your Journal can give even more valuable service by emphasizing the need of reporting hemoglobin in grams per 100 c.c.

Sincerely yours,

(Signed) Edward B. Krumbhaar,

Director of Laboratories, Philadelphia General Hospital.

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The Society maintains a Service Bureau for its members. Any clinical pathologist wishing to make a change should communicate with the Secretary.

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Kindly report any change of address to the Secretary.